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TABLE OF CONTENTS

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RENAL EXCRETION OF GENTAMYCIN IN CHRONIC PYELONEPHRITIS

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Abstract. The renal handling of gentamycin has been studied in nine patients with chronic pyelonephritis. It was found that the clearance of gentamycin was markedly below the creatinine clearance in eight patients with reduced kidney function, yielding a considerable apparent tubular reabsorption of gentamycin. In one patient with normal kidney function the amount of apparent reabsorption was negligible, giving an excretion ratio of 0.94. The dependence of log gentamycin clearance on creatinine clearance was found to be highly significant ($p < 0.001$). The possibility that gentamycin is fairly inert in relation to tubular function, as has been implied by previous studies in dog and man with normal renal function, seems unlikely. It is therefore reasonable to assume that the apparent reabsorption noted with reduced kidney function in chronic pyelonephritis may be a consequence of a reduced secretion of gentamycin due to tubular damage. An interdependence of $\log_{10}$ serum half-life ($T_{\frac{1}{2}}$) of gentamycin and $\log_{10}$ creatinine clearance (C_{cr}) was found and expressed by the regression line $Y = 1.3416 - 0.4655x$. The relationship between $T_{\frac{1}{2}}$ and C_{cr} was found to be significant ($P < 0.05$).

Although gentamycin has been available since 1962, little is known about the way in which this substance is handled by the pyelonephritic kidney. This study was carried out in order to ameliorate the situation and to demonstrate the extent of gentamycin renal clearance, tubular transport and of serum half-life ($T_{\frac{1}{2}}$) in relation to renal function.

MATERIAL AND METHODS

Patients

Nine patients with chronic pyelonephritis have been studied. In one, each kidney has been examined separately by ureter catheterization. All subjects were in a state of active infection at the time of investigation. The patients in this study included all the gentamycin-treated cases in one ward over a period of nine months. They represent a wide range of kidney function as evaluated by the endogenous creatinine clearance.

Dosage

Dosage was adjusted to body weight and serum half-life and ranged from 40 to 60 mg administered 8- to 6-hourly intramuscularly.

Sample collection

Blood specimens for serum level determinations were collected at 0–1–2–4–6 hours postprandially and separated immediately. Urine was collected through an indwelling bladder catheter at intervals of 1 to 2 hours, and diuresis was measured. The bladder was rinsed twice with saline after emptying. The serum and urine samples were stored at −20°C until they were assayed.

Gentamycin assay

Gentamycin was assayed by the cup agar two layer plate technique described previously (1). Serum samples were diluted in pooled serum from healthy blood donors, urine samples were diluted 10–20 times in Sörensen phosphate buffer of pH 6.5.

Staphylococcus aureus ATCC 6538p was used as indicator strain. The standard was prepared from gentamycin sulfate with potency 602 μg/1000 μg powder as a lot GNC 5-M 5-1 with 4.2% water furnished by the Schering Corporation, USA.

Calculations

Serum half-life values were extrapolated from least squares-calculated regression lines of the concentrations subsequent to the peak serum level.

Statistical analyses were performed according to Weber (12). The correlation coefficient (r) was calculated by the formula $r = b_{yx} \cdot s_x / s_y$, where b_{yx} is the regression coefficient of y to the independent variable x, and s_x and s_y are the standard deviations related to x and y respectively. The significance of the correlations was tested by Student's t-test based on the formula

$$t = \frac{r\sqrt{n-2}}{\sqrt{1-r^2}}$$

where n is the number of correlated observations. Only the samples collected in the phase of linear excretion (after maximum plasma levels) were employed for the calculation of gentamycin clearance and apparent tubular reabsorption.

Table I. *Excretion and reabsorption of gentamycin in nine patients with chronic pyelonephritis*[a]

Pat. no.	Creatinine clearance	Urine olume (ml/min)	Plasma[b] mean (µg/ml)	Plasma filtrable (µg/ml)	Gentamycin filtered (µg/min)	Found in urine (µg/min)	Apparent reabsorption (µg/min)	Gentamycin clearance (ml/min)	Corrected gentamycin clearance (ml/min)
1 Left kidney	23.3	0.18	2.5	1.9	43.7	16.9	26.8	6.8	9.0
	29 1	0.25	1.9	1 4	40.4	14.0	26.4	7.5	10.1
Mean	26.2								
								7.2	9.6
Right kidney	34.3	0.22	2.5	1.9	64.3	22.5	48 6	9.0	12.0
	33.0	0.26	2.3	1 7	60.4	20.4	40.0	8.9	11.8
	43 7	0.30	1.9	1.4	60.7	20.7	40 0	11.2	14.2
Mean	37 7							9 7	12.9
2.	40.0	0.76	3.5	2.6	105.0	46.0	59.0	13.1	17.5
3.	12.0	0.72	3.4	2.6	30.6	18.6	11 4	5.5	7.3
	11.0	0.67	2.9	2.2	24.2	13.4	10.8	4 7	6.1
Mean	11.5							5.1	6.7
4	108.9	3.78	3.7	2.8	302.2	287.2	15.0	77.6	103.5
	88.0	2.93	2.5	1.9	167.0	155.5	11.5	62.2	81.8
	98.6	2.63	1.5	1.3	110.9	103 7	8.2	68.5	91.3
Mean	98.5							69 4	92.2
5.	15.8	1.67	9.9	7 4	117.3	56 7	60.6	5.7	7.6
6	63.0	0.32	2.6	1.9	121.0	70.2	50.8	27 4	34 6
	78.0	0.43	1.9	1 4	108.3	64.8	43.5	35.0	46.7
	109.0	0.58	1.3	0.9	98.1	58.3	39 4	46 6	64 7
Mean	83.0							36.3	49.3
7	63.0	1.81	3.4	2.6	188.7	65 4	123.3	19.3	25 7
	37.0	2.06	2.2	1 7	61 1	22.2	38.8	10.0	13.5
	72.0	5.13	1.0	0.8	54 0	33.9	20 1	33.9	45.2
Mean	61.0							21.0	28.1
8	38.0	2.40	6.6	4.9	188 1	129.6	58.5	19.6	[illegible]2
	35.0	0.86	4.8	3 6	126.0	103.2	22.8	21.5	28.7
	44.0	0.62	3.6	2.7	117.2	82.3	34.8	23.2	30.9
Mean	39.0							21 4	28.6
9	11.0	0.08	6.0	4.5	49.5	25 0	24.5	4.2	5.6
	34.0	0.22	4 4	3.3	112.2	65.5	48.7	14.9	19.9
Mean	22.5							9.5	12.7

The calculations explained in Material and methods. All calculations to three places of decimals.
The plasma mean obtained by interpolation on the regression of the curv of serum levels at the midpoint of the period urine collection.

The difference between regression coefficients was tested by the formula

$$t = \frac{b_1 - b_2}{d}$$

where is the sum of the standard deviations of each regression divided by the degrees of freedom.

The calculation of gentamycin excretion and reabsorption followed the principles described by Dost (4). The amount assumed to be reabsorbed (R) was calculated according to $R = C \quad {}_{serr} - 1 \quad c_{u}$

C_{cr} is creatinine clearance, ${}_{serr}$ is the free, filtrable amount of gentamycin in plasma, V is the urine volume excreted per min, and is the concentration of gentamycin in the urine. Corrected gentamycin clearance was obtained by the formula

$$C_{gentamycin} = \frac{V}{serr}$$

Excretion ratio is the quotient between $C_{gentamycin}$ creatinine clearance, and equals the ratio between amount of gentamycin excreted in the urine and amount filtered by the glomeruli per min.

The serum protein binding of gentamycin is variously from 0% by cup plate technique (10) 25–30% by more accurate dialysis and ultrafiltrati procedures (2, 3) for concentrations in the lower peutic range. Accordingly 25% binding was used as basis for the above calculations I apparent tubular absorption. The results reported for the corrected gent mycin clearance and the excretion ratio may be multip by factor of 1.07 if 30% serum binding is as basis.

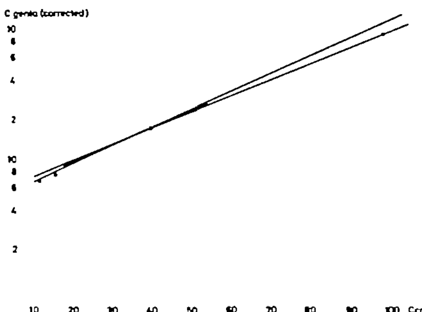


Fig. 1. The relationship between $\log_{10}$ to the corrected gentamycin clearance ($C_{genta(corrected)}$) and creatinine clearance (C_{cr}). The regression of the y on x is $Y = 0.7359 + 0.0123x$ and of the x on y is $Y = 0.06981 + 0.0132x$, where y is the $\log_{10}$ to the corrected gentamycin clearance, $r = 0.97$, $t = 11.8$ and $t(0.001, 8) = 5.04$.

RESULTS

The excretion and apparent tubular reabsorption are shown in Table I. The relation between the corrected gentamycin clearance and creatinine clearance is shown in Fig. 1. The linear regression with $\log_{10} C_{genta(corr)}$ as the dependent variable is $Y = 0.7359 + 0.0123x$. With $r = 0.97$ the correlation is highly significant ($P < 0.001$). The regression coefficient b_{yx} and r were as above for log to the uncorrected gentamycin clearance versus C_{cr}.

No relation could be demonstrated for the reabsorption ratios and the creatinine clearance $T½$ and the reabsorption ratios, the reabsorption ratios and the urine volume C_{cr} or between amount reabsorbed and the urine flow.

In some patients an increased excretion was observed with a higher urine flow whereas such a relationship was not demonstrable in others, so that no conclusion as to the influence of urine volume on the amount excreted per minute can be made in these patients.

Therapeutically important is the relationship between creatinine clearance and the serum half life which is shown in Fig. 2. The regression with $\log_{10} C_{cr}$ as the independent variable is

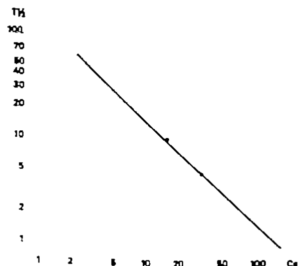


Fig. 2. Relationship between $\log_{10}$ to the creatinine clearance (C_{cr}) in ml/min and $\log_{10}$ to the serum half-life ($T½$) in hours. The line of regression is $Y = 1.3416 - 0.4655x$ with y and x the $\log_{10}$ to $T½$ and creatinine clearance respectively. $r = -0.69$, $t = 2.52$ and $t(0.05, 7) = 2.36$.

Table II. *Relationship between renal function, serum levels and ratios for elimination of gentamycin in nine patients with chronic pyelonephritis*

Pat. no.	Mean creatinine clearance (ml/mi)	Regression equation of excretion Curve = Y	Serum half-life (h)	Clearance[a] ratio (mean)	Excretion ratio (mean)
1 Left kidney	26.2	0.511–0.070x	4 6	0 27	0.37
Right kidney	37 7			0.26	0.34
2.	40 0	0.841–0.067x	5 8	0.33	0.44
3.	11.5	0.685–0 061x	4.9	0.44	0.58
4	98.5	0.745–0 116x	2.6	0.70	0 94
5	15.8	0.926–0.029x	10.1	0.36	0.48
6.	83.0	0.546–0.091x	3.3	0.44	0.59
7	61.0	0.751–0.150x	1 8	0.33	0 45
8	39.0	0 906–0 065	4.5	0.61	0.74
9	22.5	1 005–0.070x	3.9	0.41	0.55

The calculations explained in Material and methods.

$Y = 1.3416 - 0\ 4655x$ With $r = -0\ 69$ the correlation is significant at ($P < 0.05$). The validity of this regression is restricted to the range of observations.

DISCUSSION

Knowledge of how gentamycin is handled by the kidney is important due to the necessity of adjusting dosage of this drug to the renal function in order to avoid toxic blood levels. It is felt that examinations performed in patients with an active pyelonephritic process will yield information of more practical significance than results obtained with normal kidney function.

It has been demonstrated by Jao and Jackson (8) in man and by Black et al. (2) in dogs, that gentamycin clearance is approximately equal to creatinine clearance, which again is equivalent to inulin clearance or glomular filtration rate. From these observations the authors argued that there was negligible if any reabsorption or secretion of gentamycin by renal tubules. These studies were performed in patients and dogs with normal renal function. Our results in one patient (no. 4) with a creatinin clearance within the normal range are in accordance with these findings.

In the other eight patients examined, renal function was significantly reduced. The ratio gentamycin clearance to creatinine clearance was below 0 75 in all cases. *These observations might* indicate increased reabsorption of gentamycin by the tubular cells, as will b- seen from Table I. A possibly increased transport of gentamycin from the tubular lumen would most likely be due to increased back diffusion of gentamycin through damaged tubular cells.

A more likely explanation of our findings is that normally both tubular reabsorption of filtered gentamycin and secretion of gentamycin takes place through tubular cells. In renal lesions, and especially when tubular damage is present, as in pyelonephritis, there would be alterations both in tubular secretion and in tubular reabsorption of filtered gentamycin The apparent increase of tubular reabsorption might, in fact, be due to a decrease in the amount of gentamycin secreted by the tubules. Further studies are necessary to elucidate this problem. If secretion and reabsorption of filtered gentamycin take place, it is impossible to determine the amount transported by these processes without micropuncture studies or blocking of one transport mechanism.

Clearance of gentamycin does not alter with urine flow when the renal function is normal This observation has been confirmed by others (5 10) and indicates that the passive transport mechanisms are not involved in the renal transport of gentamycin through tubular cells.

Changes in renal function induced by gentamycin have usually been slight and fully reversible, although infrequently they do occur (9 10) When increase in BUN or reductions in *renal clearance have occurred they are reportedly* more frequently associated with cases where kidney function has been reduced prior to treatment (3 9 11) Although there is at present

only a strong indication of a causal relationship, our findings of an increased apparent reabsorption would provide a physiological correlate to the possibly increased incidence of nephrotoxicity in these patients. Our patients had received gentamycin for only one day prior to sample collections; therefore the changes found cannot be due to a toxic drug effect.

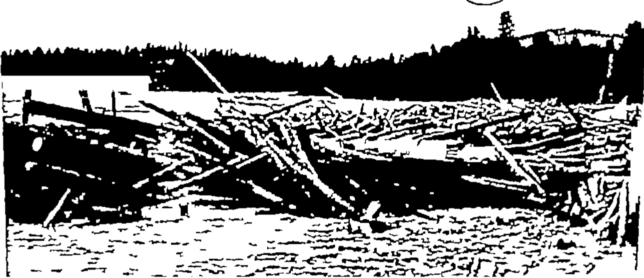
Fuchs (6) has studied changes induced by gentamycin in six patients. He reported on creatinine clearance which was mostly insignificantly reduced, but did not study changes in tubular function.

Another point which could not be usefully evaluated in this study with kidneys of impaired function was the impact of pH on the rate of gentamycin excretion. This question would be more fruitfully investigated in subjects with healthy kidneys. This matter is of considerable practical interest in view of the circumstance that the manufacturer advises alkalinization of the urine to achieve an optimal antimicrobial effect.

The variance seen in the regression between log creatinine clearance and log $T_{\frac{1}{2}}$ is not surprising in view of the measurement error inherent in both the gentamycin assay and the creatinine clearance determinations. It is remarkable that Gingell and Waterworth (7) could obtain a $r = -0.96$ for a similar correlation. However the regression coefficients found by Gingell and Waterworth and by us were not significantly different ($t = 3.45$ and $t(0.01\ \ 23) = 2.80$).

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FIBRINOLYTIC TREATMENT IN ACUTE MYOCARDIAL INFARCTION

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Abstract. A total of 426 patients admitted with symptoms of acute myocardial infarction have been treated in four hospitals. 219 were treated with streptokinase (SK), 600 000 IU or more, while 207 formed the control group. The patients were followed up as long as they stayed in the hospital, which was approximately three weeks, and by one visit to the outpatient clinic six weeks after their admission to hospital. The total mortality in the SK group during six weeks was 22 and in the control group 17 patients. If only the patients with their first episode of myocardial infarction are considered, 14 of the 156 (9.0%) in the SK group and 15 of the 163 (9.2%) in the control group died. Eight of the 40 patients with recurrent infarction in the SK group and two of the 24 in the control group died. No differences in the ECG changes were noted between the groups. The serum GOT values in the first 4 h following the infarction were higher in the SK group than in the control group in patients whose infusion had been started within the first 6 hours after the onset of chest pain. In cases with longer history no difference was observed. The ESR of the SK group fell immediately after the infusion and remained below the values of the control group for the first two weeks. The present study did not support the view that SK would be beneficial in the treatment of myocardial infarction.

Streptokinase (SK) for fibrinolytic treatment is now available in a very pure form, and the methods have been simplified to such an extent that this has become a practicable alternative in the management of thrombo-embolic diseases. Also the use of streptokinase for the treatment of myocardial infarctions has attracted great interest. A very extensive material, which gives an optimistic view of the possibility of improving by fibrinolytic treatment the prognosis for the first few weeks of myocardial infarction of less than 12 hours duration, has been published from numerous Swiss and German hospitals in cooperation (4, 5, 6). The randomisation of the material into SK-treated and control patients was however not fully satisfactory. All other material are small and usually derive from only one hospital.

The purpose of the present study was to shed additional light on the possibilities of fibrinolytic treatment in the management of acute myocardial infarction.

MATERIAL

The series consisted of patients who had been brought to the hospital with symptoms of myocardial infarction of a maximum duration of 72 h, provided that no contraindications to SK treatment were observable.

The contraindications looked for were: 1) symptoms of apoplexy; 2) systolic blood pressure exceeding 200 mmHg; 3) increased risk of haemorrhage, such as during the first three postoperative days, or in patients with thrombocytopaenia, leucaemia or haemophilia, 4) SK treatment received in the last four months, or 5) severe allergy.

Immediately on admission the patient's ECG and blood sample were taken. Numbered envelopes had been prepared. The envelope with the lowest number was taken, and the paper inside showed whether the patient was to be referred to the SK or control group.

The series was compiled in this way at the Medical Department of Turku University Central Hospital (The Turku series), at the Medical Department of the Maria Hospital of Helsinki City (The Maria series), at the Medical Department of the North Karelian Central Hospital in Joensuu (The Joensuu series), and at the Second Medical Department of the Tampere Central Hospital (The Tampere series).

The Turku and Maria series represented typical unselected patients admitted to an urban hospital for myo-

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Table I. *Number and mean age of patients in different series*

Series	SK group		Control group		
	No	Mean age (y.)	No.	Mean age (y.)	Total no. of pats.
Turku	67	58.4	60	58.4	127
Maria	42	62.0	39	55.0	81
Joensuu	32	55.3	32	54.0	64
Tampere	78	57.1	76	56.4	154
Total	219	58.1	207	56.3	426

cardial infarction. The Joensuu series was characterized by the fact that the patients often came from distant places, with the result that the most severe cases had succumbed before they reached the hospital. The Tampere series contained no very severe cases either since they were channeled to the Intensive Care Unit, the patients of which were not included in the series.

The series comprised 219 SK patients and 207 control patients, total 426 patients. It included few patients in whom the suspicion of myocardial infarction could not be verified. The total number of patients with complaints other than acute myocardial infarction was in the SK group 23 in the control group 20

The number and mean age of patients included in the series of the various hospitals are shown in Table I.

A great majority of the patients were men. Women in the SK group numbered 49 in the control group 36.

Nine SK patients and five control patients had diabetes.

The SK group included 40 patients with recurrent myocardial infarctions as compared to 24 patients in the control group. The patients who had history of angina pectoris were however, almost equally divided between

Table II. *Duration of symptoms before the initial infusion*

The Table indicates number of patients in each group

Series	Duration of symptoms (h) 0–3	4–6	7–12	13–24	25–72	Total
SK group						
Turku	32	13	7	4	3	59
Maria	1	3	2	17	16	39
Joensuu	—	1	8	11	12	32
Tampere	16	23	12	8	7	66
Total	49	40	29	40	38	196
Control group						
Turku	22	7	11	7	6	53
Maria	3	4	7	12	11	37
Joensuu	—	3	11	1	6	32
Tampere	15	16	13	14	7	65
Total	40	30	42	45	30	187

Table III. *Clinical findings in SK and control groups on admission*

	SK group	Control group
On digitalis before admission	35	16
Heart failure	36	22
Systolic blood pressure		
< 100 mm Hg	11	7
100–150 mm Hg	92	108
155–200 mm Hg	103	83
Pulse rat		
> 100/min	26	18
< 60/min	27	27
Arrhythmias		
Extrasystoles	27	25
Atrial fibrillation	9	10
Other	17	11
Pericardial friction rub	7	2

the groups (101/219 in the SK group and 94/207 in the control group).

The duration from the onset of symptoms until the beginning f the first infusion will be seen from Table II. The mean duration in the SK group of the Turku, Maria, Joensuu and Tampere series was 7 24 24 and 9 h, in the control group 12, 20, 20 and 10 h, respectively

Clinical findings in SK and control groups on admission are seen in Table III.

METHODS

All the patients of the series were first given 25 mg prednisolone intramuscularly (Di-Adreson F® Organon, Holland) in order to prevent allergic reactions to SK. This was followed by an intravenous drip infusion of 100 ml 5% glucose in one hour. In the SK group the infusion contained 600 000 units SK (Kabikinase® Kabi, Stockholm, Sweden). The patients in the Turku and Maria series were given no more SK. In the Maria series the patients were given 12 500 IU heparin subcutaneously 24 h, and 25 000 IU 36 h, after the institution of the infusion (Heparin Subcutan® Star Tampere, Finland).

For the Joensuu and Tampere series the clottable fibrinogen was determined by the Adamis test one hour after the first SK infusion had been completed (2). If the value exceeded 50 mg/100 ml, further 250 000 IU were given within half an hour in 100 ml 5% glucose, and the fibrinogen was determined again an hour after the completion of the infusion. This continued until the result was below 50 mg/100 ml, after which the test was repeated every 8 h, for the Joensuu series only in the daytime. Once the fibrinogen content again exceeded 80 mg/100 ml, further 250 000 IU were given in 4-hour drip infusion of 500 ml 5% glucose. In this way an attempt was made to keep the value below 80 mg/100 ml for 48 h. Table IV shows that in most patients

of the Tampere and Joensuu series this was achieved either by the initial 600 000 IU dose alone or with the initial dose plus 1 3 additional doses of 250 000 IU

All patients were given oral anticoagulant treatment from the day of admission and for a minimum of six weeks. The drugs used were either phenylindanedione (Trombol® Star, Tampere, Finland, Tromobex® Leiras, Turku, Finland) or sodium warfarin (Marevan® Orion, Helsinki, Finland). The treatment was started with standard dose, and from the third day onwards the dose was adjusted by means of the Quick test using Thrombokinase Geigy (Geigy Basle, Switzerland) and control curve the manufacturers had plotted using adsorbed plasma. An exception was the Maria series, where the dose was adjusted by means of the P & P value. Quick values from 15 to 30% and P & P values from 10 to 20% were considered to be the therapeutic level.

The patients were followed up during their hospitalization, which was usually about three weeks, though in the Turku series slightly less, and at an outpatient visit six weeks after admission. Table V lists various laboratory tests used for the observation of the patients.

Before the institution of the first infusion the Tampere and Joensuu series (218 patients) were subjected to bedside TID test performed as instructed by the manufacturers of the reagent used (Kabi). The TID results were, with one exception, less than 600 000 IU

RESULTS

Mortality

Table VI shows the mortality rates of the various series classified according to the time interval between the onset of pain and initiation of treatment.

Death ensued within the 24 hours following the beginning of the initial infusion in eight cases of the SK group and six cases of the control group. Later in the course of the follow-up period,

Table IV *The amounts of SK given to the patients of the Tampere and Joensuu series*

Column A indicates how many infusions containing 250 000 IU of SK were given to the patient in addition to the initial infusion which contained 600 000 IU

A	Total amount of SK given (IU)	No. of pats.
0	600 000	40
1	850 000	37
2	1 100 000	20
3	1 350 000	9
4	1 600 000	—
5	1 850 000	1
6	2 100 000	1
7	2 350 000	2
	Total	110

Table V *The tests used in the follow-up of the patients, and their intervals calculated from the institution of the first infusion*

The GOT values determined in Wroblewsky units except in the Turku series in which microunits were used. Th[illegible] CPK determined only in the Tampere series. The ECG taken [illegible] three bipolar and three unipolar limb leads and [illegible] chest leads

Interval	Tests
0 h	GOT CPK, ECG BSR, leuc
6 h	GOT CPK, ECG
12 h	GOT CPK, ECG
24 h	GOT CPK, ECG BSR, leuc
3rd d.	GOT CPK, ECG BSR, leuc
5th d.	ECG
7–10th d.	GOT CPK, ECG BSR leuc
14–21st d.	ECG
42nd d.	ECG BSR, leuc

14 patients of the SK group [illegible] 11 [illegible] f the co[illegible] trol group died

Eight deaths in the SK grou[illegible] invol[illegible]ed patien[illegible] with a recurrent infarction, as [illegible]d t[illegible] the control group The mortal[illegible] rat[illegible] with their first myocardial infa[illegible]ction 156 (9.0%) in the SK group [illegible]nd 15 (9.2%) in the control group Among pat[illegible] with recurrent infarctions the mortality [illegible] the SK group was eight of 40 and in the con[illegible] group two of 24

There was no appreciable differenc[illegible] in th[illegible]

Table VI. *Mortality rates by groups based on the interval between the onset of the symptoms of the present myocardial infarction and the institution of the initial infusion*

The Table lists the number of deaths per group

Series	Duration of symptoms (h) 0–3	4–6	7 12	13–24	25–72	Total
SK group						
Turku	6	2	2	—	—	10
Maria	—	1	—	3	3	7
Joensuu	—	—	—	1	—	1
Tampere	1	—	2	—	1	4
Total	7	3	4	4	4	22
Control group						
Turku	1	1	3	1	2	8
Maria	1	—	1	1	1	4
Joensuu	—	1	—	—	—	1
Tampere	3	—	—	1	—	4
Total	5	2	4	3	3	17

and –

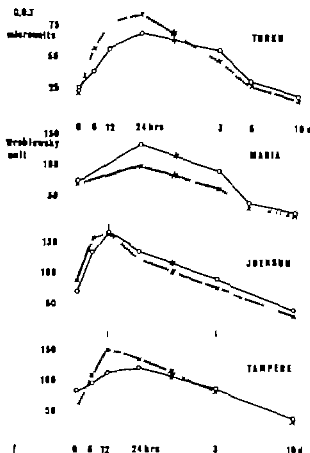


Fig. 1 Serum glutamic oxalacetic transaminase (GOT) in the series of the different hospitals. In the Turku series *GOT was determined in microunits, in the other* series in Wroblewsky units. -- SK group: O—O control group.

causes of death and autopsy findings between the SK and control groups. Ruptured heart was found in five patients of the SK and two of the control group, all with their first infarction. In one case of the SK group an additional finding was cerebral embolism, in a control group case pulmonary embolism.

The need for analgesics

To alleviate pain, the patients were given 75 mg pethidine chloride at a time (1.5 ml Pethidin® Star Tampere, Finland) The purpose was to make the number of the injections proportionate to the sensations and complaints of pain. In the first 48 h the SK group patients were given an average of 2.9 injections, the control group patients 3.1 injections.

Clinical course

Most patients recovered without any particular complications. Within the six weeks there were only two relapses in the SK, and one in the control group. In addition there were two apoplectic insults in both groups. One in each group was transient.

Electrocardiography

In the Turku series the development of ST deviation was determined as follows. From the ECG taken on admission the ST segment change was measured in mm from the baseline in the lead where the deviation was greatest. The ST changes were measured from the same lead again on the second and the third days. The mean ST deviations in the SK group were 2.7 1.7 and 1.7 mm, and in the control group 3.0 2.0 and 2.0 mm. Hence no differences in the change of the ST segment were noted between the two groups.

Glutamic oxalacetic transaminase (GOT)

The GOT values 6, 12 and 24 h after the infusion seemed to be somewhat higher in the SK than in the control groups of the Turku and Tampere series, which included a large proportion of infarctions with short duration of symptoms (Fig. 1) No such difference was observed in the Joensuu series. In the Maria series the GOT values were not determined 6 and 12 h after the infusion.

To examine the influence of the duration of symptoms before the initial infusion, the Turku, Tampere and Joensuu series were divided into four parts according to the time interval in hours (Fig. 2) Both in the combined Tampere–Joensuu and in the Turku series, in the group with duration of symptoms less than 3 h, the GOT change from 0-hour value to 6-hour and to 12-hour values was significantly greater in the SK than in the control group ($p < 0.05$) (Sten Martinelle, M.Sc.) The same was true of the GOT change from 0-hour value to 6-hour value in the group where the time interval was 4–6 h (Fig. 2)

Among those with a longer history no such difference was observed.

Creatinine phosphokinase (CPK)

The CPK was examined only in the Tampere series (Fig. 3) It seems that in patients who had the attack of chest pain less than 3 h before the

treatment was begun, the CPK 6 and 12 h after the initial infusion was higher in the SK group than in the control group, whereas in those who had had the pain for a longer time it was lower than in the control group throughout the first 4 h. Since the series was small, these observations lack statistical significance (Sten Martinelle, M Sc.).

Blood sedimentation rate (BSR)

Fig. 4 shows the changes in BSR in the various series during the follow-up period. In the SK group the BSR was distinctly lower than in the control group on all measurements during the first two weeks following the initial infusion. In the first 4 h after the infusion the BSR even fell

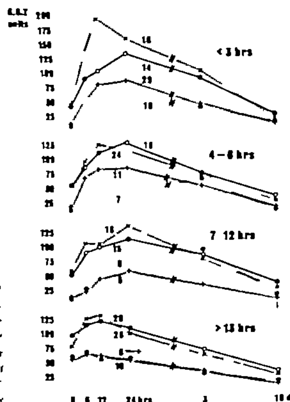


Fig. 4. Serum GOT classified according to the duration of symptoms. Duration of symptoms before institution of the initial infusion, from top to bottom. 0–3 h, 4–6 h, 7–12 h and 13–7? h. — SK group of the combined Tampere and Joensuu series, O—O control group of the combined series above +—+ SK group of the Turku series; — control group of the Turku series. The plotted values are mean values. The figure beside each curve indicates the minimum number of values from each each of the first four mean values of the curve were calculated

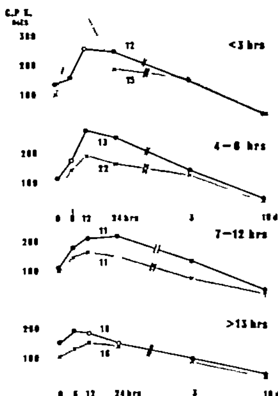


Fig 3 Creatinine phosphokinase (CPK) in the Tampere series, classified according to the duration of symptoms. Duration of symptoms before the initial infusion, from top to bottom: 0–3 h, 4–6 h, 7–12 h and 13–72 h. — SK group; O—O control group. For the figures next to the curves, see legend of Fig. 2.

instead of increasing. No mutual differences were noted between the series of the different hospitals.

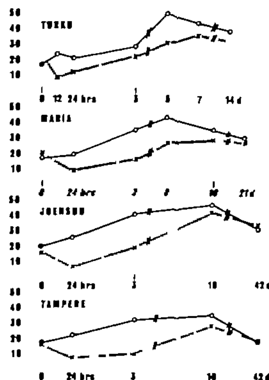
Leucocytes

In the course of the follow-up period, the leucocyte values revealed no differences between the SK and control groups.

Complications

Microscopic haematuria was the most frequent haemorrhagic complication recorded: seven cases in the SK and three in the control group. Furthermore, two gingival haemorrhages, one haematemesis, and two instances of haemorrhage or bruises at the site of injection were recorded in the SK group Most haemorrhages were very slight, and caused the SK or anticoagulant therapy to be discontinued in only three cases of the SK group for gingival haemorrhage – h and for

(cont.)



4. Blood sedimentation rate in the series of the different hospitals. — SK group; ○—○ control group.

haematemesis 24 h after the initial infusion, and for haematuria on the 5th day of treatment.

Among complications other than haemorrhagic, transient proteinuria attracted attention in the Turku series. This occurred after the initial infusion in 32 patients of the SK group but only in six patients of the control group. The other series were not studied systematically for this complication. Cases of glucosuria numbered 7 in both groups. Cerebral insults occurred in two cases in both groups during hospitalization. In the first two days of treatment there were two vascular shocks in the SK group and three in the control group. In addition there was one anaphylactic shock in the SK group immediately after the first infusion had begun. Chills were noted in 17 patients of the SK group and in seven of the control group. Three patients of the SK group suffered from nausea during the first two days of therapy.

The highest mean temperatures recorded during the first week of treatment were exactly the same for the SK and control groups in the Turku (37.7 C) and Maria series (38.2 C, rectal temperature) and almost the same in the Tampere series (SK group 37.5 and control group 37.4 C). In the Joensuu series it was 37.8 C in the SK and 37.4 C in the control group. In the SK group there were 26 patients whose temperatures exceeded 38.5 C during the first week, and in the control group there were 24. The rise in temperature of the patients treated with SK could therefore usually be ascribed to their disease.

DISCUSSION

In the SK treatment of the present series small doses were used. It should be noted however that the same dosage has led to as good results as a higher dosage in peripheral arterial thromboembolism and pulmonary embolism (3). Taking into account that the thrombotic mass involved in myocardial infarction is much smaller than the thrombotic mass of the peripheral emboli the present negative therapeutic result can hardly be attributed to too small a dose.

There was no difference between the groups in the mortality rates, which in the first 24 h, and also after the first 24 h, were the same in both groups. The SK group contained slightly more severe cases than the control group but the main differences were due to the coincidence that the group contained more recurrent infarctions than did the control group.

While the present material was being collected, a similar material was also collected by certain Belgian and German hospitals in cooperation (1). With the use of a very high dosage (1 250 000 IU in the first half hour and subsequently 100 000 IU/h for 72 h) the mortality rate in the SK group of 81 patients (18 deaths in 6 weeks) was no lower than that in the control group of 83 patients (14 deaths). Even with such high doses, the result of the therapy was not favourable. For patients with a history of pain lasting less than 1 h the result was the same: the mortality rate in the SK group was nine out of 54 and in the control group eight out of 45.

In the present series, too, no differences to accord with the duration of symptoms before admission were seen in mortality rates between the SK and control groups. The reactions in certain laboratory tests, however revealed interesting differences.

The reaction of BSR to SK treatment in all the hospitals was evident, irrespective of the time interval between onset of pain and institution of treatment. A natural explanation is, perhaps, that the fibrinogenolysis produced by SK eliminates the additional fibrinogen caused by the infarction and, therefore, for some time, normalizes the BSR which already had been on the increase. The fact that the reaction did not correlate with the duration of the history of the infarction would suggest that the reaction does not reflect any thing that is taking place in the myocardium

The GOT change after SK treatment seemed to be directly affected by the duration of symptoms before the institution of treatment. Körtge et al. (4) have presented the following explanation of the acceleration in the GOT rise during first few hours after SK treatment. SK produces a recanalization thanks to which the enzymes liberated from the myocardium are more y carried into the blood circulation. The series gave no evidence of the other phe described by Körtge, viz. that the GOT also fell more rapidly in the SK group after the initial rise. The fact that the GOT change due to the influence of SK was no longer recorded in the group of patients with a history of pain for more than 4 h as clearly as in the group with a history of pain for less than 3 h, and not at all in that with pain for more than 7 h, would suggest that recanalization could no longer be achieved.

The CPK changes were studied in such a small group that the findings are rather a suggestion than evidence of its reactions in the various groups of the history of pain.

It has earlier been found (5) that patients treated with SK showed a more rapid development of the final infarction signs in the ECG than control patients. Also the reversal of infarction signs was more rapid in SK patients than in controls. This finding could not be confirmed in our series, in which no differences in the change of the ST-segment were noted between the groups.

The number of haemorrhagic and other complications recorded in the SK group was slightly higher than in the control group It cannot be excluded, however that the hospital personnel react more readily to the symptoms of the SK than of the control group patients. The only exception is proteinuria which, judging from hospital records analysed after the series had been compiled, had had a considerably higher incidence in the SK than in the control group Whether this could be due to the split products produced by fibrinolysis and fibrinogenolysis, which may have been secreted into the urine and have caused a positive protein reaction, must be ascertained by future studies.

It may be concluded that nothing in the present series indicates that SK treatment would be beneficial in cases in which the history of chest pain is longer than 7 h There are certain signs to suggest that SK therapy of completely fresh cases might produce the recanalization of a coronary artery which was already blocked, but no clinical benefits from this recanalization could be demonstrated.

It remains to be seen whether in the future with well chosen dosage and a skilful selection of cases, a use may be found for SK in the management of acute myocardial infarction.

ACKNOWLEDGEMENT

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"Comparative study of a long acting quinidine preparation and quinidine sulphate in chronic atrial fibrillation

Török E., Bajkay G. Gulyás, A. & Makláry E. Pharmacol Clin. 2:90 1970.

During a period of 15 months a comparative study between Kinidin Durules® and ordinary quinidine sulphate tablets was performed. 26 patients with atrial fibrillation were DC-reverted and subsequently treated with quinidine as maintenance therapy. The aim of the study was to obtain answers to the following questions

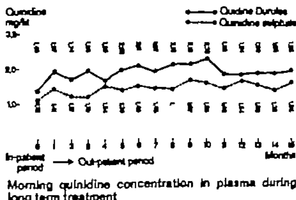
- Is there any difference in the therapeutic value of Kinidin Durules® and ordinary quinidine sulphate tablets in the maintenance of sinus rhythm after DC-shock in patients with atrial fibrillation?
- Which of the two preparations gives the least side effects?
- Is there any difference between the two presentations in the maintenance of stable and sustained plasma quinidine levels?

The study gave the following results

- In the maintenance of sinus rhythm the two preparations were found to be equivalent.
- Quinidine sulphate tablets caused side effects more frequently than the long-acting Kinidin Durules® did. Gastrointestinal symptoms were observed in five patients in the quinidine sulphate group but only in one in the Durules® group.
- With twice daily administration of Kinidin Durules® a more stable plasma quinidine level was maintained compared with quinidine sulphate tablets administered four times daily. The difference in the morning levels was particularly striking. With Kinidin Durules® the level was higher and there were no differences between morning and afternoon levels, see figure below

The authors conclude

Our study supports the superiority of the long-term treatment with Kinidin Durules® as compared to quinidine sulphate for the maintenance of sinus rhythm. The antiarrhythmic potency of the Durules® is equal to that of the quinidine sulphate while the need of only two doses daily is more favourable for the patient and ensures a more stable plasma quinidine level. That in turn might explain the less frequent occurrence of side effects and especially of gastrointestinal complaints.



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Acta med. scand. Vol. 189 pp. 15–19 1971

EFFECT OF DIPHENYLHYDANTOIN ON THE METABOLISM OF DICOUMAROL IN MAN

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Abstract. The effect of diphenylhydantoin on dicoumarol metabolism has been studied. Diphenylhydantoin decreases serum dicoumarol and increases prothrombin-proconvertin concentration in patients receiving dicoumarol orally. The same effect was found when dicoumarol was given intravenously. The decrease in serum dicoumarol was evident after few days of diphenylhydantoin treatment, but after withdrawal an increase in serum dicoumarol was not seen until 2–3 weeks later. Half-lives f intravenously injected dicoumarol showed inconsistent changes following diphenylhydantoin treatment. Diphenylhydantoin probably acts by inducing the drug metabolizing enzyme system.

Several drugs are known to influence the effect f coumarin anticoagulants on the prothrombin time. The danger of giving sulfonamides and broadspectered antibiotics to patients treated with anticoagulants is wellknown. The mechanism of this effect is considered to be a suppression of the intestinal bacterial flora which normally produces a certain amount of vitamin K_1 (17 28). Phenyramidol, disulfiram, chloramphenicol and methylphenidate have been shown to inhibit the metabolism of the coumarins (4 14, 26, 29).

Also phenylbutazone and oxyphenbutazone are known to enhance the effect of peroral anticoagulants. In these cases the mechanism is considered to be a displacement of the anticoagulant from its protein-binding (1). Salicylate is known to have a direct hypoprothrombinemic effect (20). Several other drugs have been reported to increase the prothrombin time in anticoagulated patients, but conclusive experiments to elucidate the mechanism are not available. This applies to *d* thyroxine, clofibrate, anabolic steroids, benziodarone and quinidine (15 24 27).

Other drugs, however have been shown to decrease the action of anticoagulants. The mechanism is supposed to be an induction of the enzymatic hydroxylation of the coumarins in th liver. Most experiments refer to phenobarbitone (7 1) but also other barbiturates (2, 22), glutethimide, chlordiazepoxide, diazepam (10) chloral hydrate (8) and griseofulvin (9) have been shown to stimulate the metabolism of coumarins.

We have shown (16) that dicoumarol is potent inhibitor of diphenylhydantoin metabolism. The mechanism of this effect might possibly be a competition between the two drugs for the enzyme apparatus which hydroxylate their ring structure. According to this explanation it should be expected that diphenylhydantoin, on the other hand, would inhibit the metabolism f dicoumarol. The present work was performed to test this idea.

METHODS

The prothrombin-proconvertin concentration f the plasma (PP%) was determined by the method described by Owren and Aas (23) Diphenylhydantoin added in vitro did not influence the analyses.

Serum dicoumarol as determined by the method described by Axelrod et al (3) Diphenylhydantoin did not affect the results.

Serum diphenylhydantoin was determined ad modum Dill et al (13)

Dicoumarol used for intravenous injection as immediately before injection, dissolved in sterile solution of propylene glycol, ethanol and sodium hydroxide in water The concentration of ^{14}C-dicoumarol as determined in liquid scintillation counter using solution of 3 5 g PPO, 0 15 g POPOP 90 g naphthalene and 900 ml of dioxane.

The half-life of dicoumarol in serum as ermined

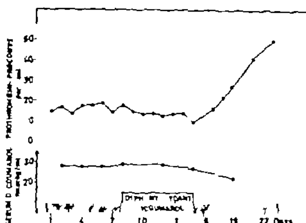


Fig 1 Mean values of serum dicoumarol and prothrombin-proconvertin % before, during and after diphenylhydantoin treatment in six dicoumarol-treated persons.

by giving an intravenous dose f 170–225 mg dicoumarol and measuring serum dicoumarol for a period of 12 72 hours after the injection. In two patients (one receiving only 45 mg dicoumarol) 15 μCi dicoumarol labelled with ^{14}C at the methylene bridge was added to the carrier dose of dicoumarol and the radioactivity was measured in extracts f plasma from blood samples taken as mentioned above. Thin layer chromatography was used to confirm that all the radioactivity was in the dicoumarol spot. The two ways of determining the dicoumarol half-life gave identical results.

The LKB 6300 A ultrafilter equipment was used for determination of non-protein-bound dicoumarol. Dicoumarol and tracer amounts of ^{14}C-dicoumarol were added to 25 ml serum adjusted to pH 7 4 by phosphate buffer giving a concentration of 30 μg dicoumarol per ml. The radioactivity in 200 μl ultrafiltrate was expressed in per cent of the radioactivity in 200 l of the serum solution. Free dicoumarol was determined in serum samples without adding diphenylhydantoin and with diphenylhydantoin concentrations of 15 and 30 μg/ml.

All the patients examined were volunteers. None received other medicaments during the period of investigation and none of the patients showed signs of renal or hepatic disease or suffered from congestive heart failure. Their general condition was good and unaltered during the investigation.

RESULTS

Fig. 1 shows the mean values of PP% and serum dicoumarol from six dicoumarol-treated persons before, during and after seven days treatment with diphenylhydantoin 300 mg daily A constant daily dose of dicoumarol (40–160 mg daily) was given during the whole period of investigation. No significant changes in the PP% are seen until three days after withdrawal of diphenylhydantoin in the following five days PP% increased from 20 to 50% Serum dicoumarol decreased from 29 μg/ml on the fifth day of diphenylhydantoin treatment to 21 μg/ml five days after withdrawal of the diphenylhydantoin. The mean value of the maximal concentrations of serum diphenylhydantoin was 18 μg/ml.

Another four volunteers were studied for a longer period and Fig. 2 gives the results from one of these persons who received a constant dose of 60 mg dicoumarol per day during the whole period of investigation When stable values of PP% and serum dicoumarol were obtained, diphenylhydantoin was given in the following $6^1/_2$ weeks. In the first week the dose was 300

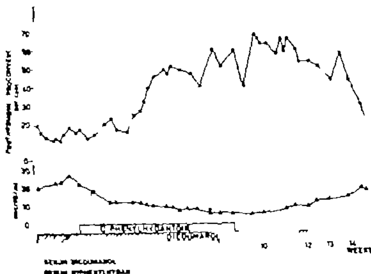


Fig. 2 Changes in serum dicoumarol and prothrombin-proconvertin % during and after diphenylhydantoin treatment in one dicoumarol-treated person.

mg daily but this dose had to be reduced to 100 mg daily in the following weeks. The values of serum diphenylhydantoin obtained are shown. Serum dicoumarol decreased from 70 μg/ml at the beginning of the diphenylhydantoin treatment to 5 μg/ml at the time of withdrawal of the latter. Two weeks later serum dicoumarol began to increase, and a value of 18 μg/ml was found 3 1/2 weeks later. The PP% increased after about two weeks of diphenylhydantoin treatment from 20% to 70% and a decrease was seen two weeks after withdrawal of diphenylhydantoin, the pre treatment value being obtained after 5 1/2 weeks. Three other patients were examined in a similar way. One patient showed exactly similar changes in serum dicoumarol and PP% as the patient mentioned above (Fig. 2). The two other persons showed also a significant fall in serum dicoumarol but only a slight rise in PP% during diphenylhydantoin treatment, and they were not followed after withdrawal of diphenylhydantoin.

Two volunteers were given an intravenous dose of 30 mg dicoumarol daily for one month and Fig. 3 shows the variation in serum dicoumarol before, during and after one week's treatment with diphenylhydantoin perorally in one of these persons. A few days after the diphenylhydantoin treatment was started, a decrease in serum dicoumarol was observed. This fall continued until six days after withdrawal of diphenylhydantoin, when serum diphenylhydantoin had reached low values. Six days later serum dicoumarol again increased. The second person receiving dicoumarol

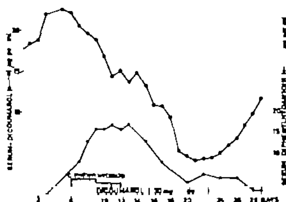


Fig. 3. Changes in serum dicoumarol caused by diphenylhydantoin treatment in person given dicoumarol intravenously.

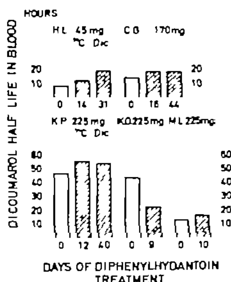


Fig. 4. Values of dicoumarol half-life in blood before and during diphenylhydantoin treatment.

intravenously showed a similar decrease in serum dicoumarol and an increase in PP% during diphenylhydantoin treatment, but was not followed more than a few days after withdrawal of diphenylhydantoin.

In five patients the half-life of intravenously injected dicoumarol was determined before and after treatment with diphenylhydantoin. The results are shown in Fig. 4. Dicoumarol half-life in blood increased in one person, decreased in one, and in the three other persons a hardly significant increase was found even after six weeks treatment with diphenylhydantoin. The concentration of serum diphenylhydantoin varied between 10 and 18 μg/ml and no correlation was found between the blood level of diphenylhydantoin and the changes in dicoumarol half-life. To test the reproducibility of the half-life determinations, two persons were given 220 mg dicoumarol intravenously twice with ten days interval and without receiving any other medicaments. The values obtained were 20 / and 17 hours, and 24 and 21 hours, respectively.

Determination of ultrafiltrable dicoumarol in serum gave value of 0.70% of total dicoumarol. After adding diphenylhydantoin to concentrations of 15 and 30 μg/ml, values of 0.78 and 0.74% respectively were obtained.

DISCUSSION

No previous reports are available concerning the influence of diphenylhydantoin on the metabolism of dicoumarol The purpose of the present study was to test whether competition phenomena might cause a slowing down of dicoumarol metabolism when diphenylhydantoin was given simultaneously On the contrary we have found that the administration of diphenylhydantoin decreases the serum dicoumarol values and increases the PP% in plasma in patients receiving a constant oral dose of dicoumarol.

Recent work has shown, however that diphenylhydantoin accelerates the biotransformation of some drugs in man by inducing the metabolizing enzyme system. Conney (6) found that diphenylhydantoin stimulates the 6-beta-hydroxylation of cortisol. Davies et al. (11) showed that the removal of D.D.T residues seems to be enhanced by prolonged treatment with diphenylhydantoin. Furthermore, animal experiments have shown increased metabolism of hexobarbital, pronarcon, meprobamate, strychnine, pentobarbital and metyrapone during diphenylhydantoin treatment (18 19 25 30)

Our findings that the decrease in serum dicoumarol was evident after a few days of diphenylhydantoin treatment, and especially that an increase in serum dicoumarol was seen only 2–3 weeks after withdrawal of diphenylhydantoin, are consistent with the explanation that diphenylhydantoin acts primarily as a stimulator of dicoumarol metabolism

Failure of dicoumarol absorption caused by diphenylhydantoin might be followed by similar alterations in serum dicoumarol and PP% but the delayed effect following withdrawal of diphenylhydantoin on serum dicoumarol levels is difficult to explain by a change of dicoumarol absorption from the intestines. Recently O'Reilly and Aggeler (22) suggested that heptobarbital—a well known enzyme inductor—might possibly interfere with the intestinal absorption of dicoumarol. The result of the experiments (Fig 3) in which the dicoumarol was given by the intravenous route gives no support for any major effect of diphenylhydantoin on dicoumarol absorption.

To further elucidate the mechanism of diphenylhydantoin action on dicoumarol metabolism the half-life of dicoumarol in blood was determined before and during diphenylhydantoin treatment. The results showed inconsistent changes in dicoumarol half-life even after prolonged diphenylhydantoin treatment. The half-life decreased in only one of five persons. These results are not in accord with the results of the serum dicoumarol and PP% determinations. O'Reilly and Aggeler (22) found a similar discrepancy in their studies of the effect of heptobarbital on serum dicoumarol.

In the half-life determinations, evidently no dicoumarol was given in the interval between the two intravenous injections of the half life doses of dicoumarol. This is in contradistinction to the experiments showing a decrease in dicoumarol concentration in blood when both drugs were given simultaneously A prerequisite for induction of the enzyme apparatus metabolizing a certain drug might, consequently in some cases be that the drug and the inducing medicament are given together for a period.

In our experiments a possible competition of the two drugs for the hydroxylating enzyme apparatus may even further complicate the picture. This effect might possibly explain the increas. in half-life observed in some of the patients. The above mentioned experiments and other observations of ours suggest that a determination of half life values may not always elucidate the mechanism of an interaction between different drugs, a problem which will be dealt with more thoroughly in a later paper (5)

The ultrafiltration experiments show that our results are not complicated by phenomena of displacement of dicoumarol from its protein binding by diphenylhydantoin

The practical outcome of our experiments and observations evidently is that dicoumarol and diphenylhydantoin should not be given simultaneously without a great deal of caution The risk of bleeding will be considerable in patients receiving both dicoumarol and diphenylhydantoin If diphenylhydantoin is discontinued without reducing the dose of dicoumarol. Fatal outcomes due to such an effect have been reported (8, 21) when other inducing drugs have been discontinued in patients treated with anticoagulants.

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THE EFFECT OF PAPAVERINE, LIDOCAINE AND HEPARINE ON THE VASCULAR RESISTANCE IN HYPOTHERMIC KIDNEY PERFUSION

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Abstract. The effect of papaverine, lidocaine and heparine on the vascular resistance in hypothermic perfused rat kidneys has been investigated in one hundred experiments, using low molecular weight dextran 10% with NaCl 0.9% (Rheomacrodex® MW 40000) as perfusate medium. The vascular resistance at the start of perfusion is increased due to vasospasm, arising in connection with dissection of the renal pedicle and perfusion with cold fluid, but decreases during continued perfusion. Papaverine and lidocaine, added to the perfusate medium in concentrations of 5 and 100 mg% respectively reduce the vascular resistance significantly. Heparine in the perfusate medium increases the vascular resistance, but this effect can be neutralized by addition of papaverine. Heparinization of the animals before removal of the kidneys reduces the vascular resistance at the start of the perfusion. A combination of heparinization of the animals before removal of the kidneys and addition of 5 mg% papaverine or 100 mg% lidocaine to the perfusate medium results in the lowest vascular resistance at the start of perfusion and, therefore, probably yields the best conditions for rapid and uniform perfusion.

Hypothermic extracorporeal perfusion with different perfusate media has found an increasing use in kidney preservation (2, 5, 7, 8).

On the assumption that the perfusion is started within a few minutes after removal, the ease with which a kidney is *perfused depends partly on* vasospasm developed before the start of the perfusion, partly on vasospasm in connection with the cold perfusate medium.

Vasospasm in the kidney before removal has been described in connection with surgery involving dissection on the renal pedicle (6, 10, 11, 12). *This may* decrease the renal blood flow and the urinary output. Anuria for up to 45 min has been described (11). In preservation experiments with pig kidneys we have observed pronounced vasospasm of the renal artery during dissection on the renal pedicle and simultaneous measurements of the renal blood flow using Xe^{133} often revealed values of nearly zero (9). Changes in the distribution of the renal blood flow have been described, and especially the supply to the renal cortex seems to suffer by surgical manipulation of the kidney (4, 6, 12). Periods of hypotension before removal of the kidney also seem to increase the tendency to vasospasm (1, 4).

The effect of hypothermia on the vascular resistance has been described by Bickford and Winton (3) in experiments with perfusion of dog kidneys, using defibrinated blood as perfusate medium. Apart from a transient vasoconstriction due *to sudden cooling no marked changes other than* those due to changes in the viscosity were found.

Varying degrees of vasospasm do change the rate of perfusion and consequently affect the rate of cooling of the kidney. Furthermore they may change the intrarenal distribution of the perfusate medium, resulting in insufficient perfusion of parts of the kidney. Therefore vasodilators are often added to the perfusate. Moreover heparine is widely used in the perfusate to prevent intravascular coagulation, which may interfere with fast and uniform perfusion of the organ.

Apparently no systematic investigation has been made of the effect of vasodilators and heparine on the vascular resistance at the beginning of hypothermic kidney perfusion. It is the purpose of this work to investigate the effect of papaverine (Papaverini sulfas NFN), lidocaine (Lidocaini chloridum NFN) and heparine (Heparinum NFN) on the vascular resistance in experiments with hypothermic perfusion of rat kidneys.

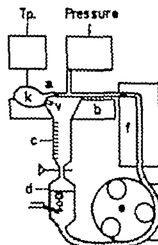


Fig. 1 Schematic representation of the perfusion circuit. For further details see text.

MATERIAL AND METHODS

The experiments were performed in perfusion apparatus which allowed hypothermic perfusion with continuous control of arterial pressure, flow rate and temperature (Fig. 1).

Before the start of the perfusion the apparatus was filled with the perfusate medium, 20 ml low molecular weight dextran 10%, with NaCl 0.9% (Rheomacrodex® MW 40000). Vasodilators and heparine were added to the perfusate medium in varying amounts. Within the experimental period the total volume of the perfusate passed the kidney about three times. The kidney (*k*) was placed on a disc (*b*). Through a funnel-shaped opening in the disc the perfusate flowed freely from the vein ( ) to flowmeter (*c*) and an oxygenator (*d*). An occlusive roller pump (*e*) (Watson-Marlow MHRE) pumped the perfusate through a cooler (*f*) to the renal artery (*a*). The arterial pressure was measured about one cm from the hilum by means of a pressure transducer (Harvard Model 375) and was registered on a chart mover (Harvard Model 450). The roller pump had a flow capacity from 0 to 10 ml per min, and each tube was calibrated before use, allowing the flow to be adjusted before the start of the perfusion. To control this adjustment, the flow was measured twice during the perfusion by means of the flowmeter. The temperature was followed by means of a thermistor (Type ISC Ellab) connected to an electric thermometer (Type TE 3 Ellab).

The material includes one-hundred rat kidney perfusions divided into ten groups with ten perfusions in each group. The experimental conditions were varied by changing the concentrations of vasodilators and heparine in the perfusate medium, and in some cases by heparinization of the animals before removal of the kidney. Table I shows the variations in the experimental conditions in the ten groups.

When the perfusion apparatus was ready for use, the rats were anaesthetized by intraperitoneal injection of inj. pentymal: NFN 25 mg 100 g body weight. The abdomen was opened and the left kidney left renal artery and vein with adjacent parts of the aorta and the caval vein were separated, following which the aorta was ligated proximal to the renal artery and the renal vein ligated near the caval vein. Then the kidney was removed by cutting first the renal vein and after this the aorta at distance of 3 mm above and below the origin of the renal artery. The kidney was then placed in a Ringer solution at room temperature and the aorta was cut up in order to visualize the opening of the renal artery. After having been wiped the kidney was weighed, and on the basis f the weight the roller pump was adjusted to give a flow corresponding 0.5 ml/g kidney weight/min. Then the kidney was placed in the perfusion apparatus and the renal artery was cannulated. The thermistor was placed 2 3 mm inside the kidney and the perfusion was started. The time from ligation of the aorta to the start f the perfusion was seven minutes. Two hours later the perfusion was stopped, and the kidney was wiped and weighed again.

Table I *A summary of the varying conditions in the ten groups of rat kidney perfusions*

Group	Concentration in the perfusate medium: Papaverine (mg/100 ml)	Lidocaine (mg/100 ml)	Heparine (IU/100 ml)	Heparinization 5 min before removal (IU)
1	0	0	0	0
2	0	0	5 000	0
3	0	0	0	500
4	1	0	0	0
5	5	0	0	0
6	5	0	5 000	0
7	5	0	0	500
8	40	0	0	500
9	0	50	0	500
10	0	100	0	500

RESULTS

Table II shows the changes of the arterial pressures during the perfusion in the ten groups. Table III shows the means and the S.D.s of the measured values of the flow rate, the temperatures after ten minutes perfusion and the weight increases after two hours perfusion.

The measured flows varied as shown in Table III The consequence of this, when the arterial pressures in the different groups are compared, will be discussed later

The temperature varied between 8.1 to 9.6 C, and the differences were statistically significant between some of the groups. However these small variations in the temperature had no in-

Table II. *The arterial pressure in the ten groups of rat kidney perfusions at various times during the perfusion. Each value is the mean of ten perfusions. The S.D s are stated*

Group no	Arterial pressure (mm/Hg) 1 min	5 min	10 min	15 min	20 min	40 min	100 min
1	162±21	149±21	102±33	70±28	54±20	40±11	54±11
2	159±44	137±45	123±47	115±45	96±32	69±15	71±20
3	117±34	107±39	106±48	91±36	82±35	62±14	62±13
4	110±31	87±33	68±17	55± 9	46± 8	40± 5	45± 4
5	120±25	72±17	48±11	41± 7	39± 5	40± 5	50±11
6	150±41	78±26	53±18	46±17	43±13	40± 6	48±17
7	81±26	53±21	44±11	42± 6	40± 5	40± 6	53±12
8	132±12	96±15	65±10	56±16	51± 7	51± 6	67±14
9	129±37	92±25	76±19	65±15	55± 9	47±10	51± 8
10	129±22	68±12	55±11	51±11	48±10	49±13	58±17

fluence on the arterial pressure. A comparison between eight perfusions at an average temperature of 7 7 C, and twelve perfusions at an average temperature of 9 0 C ($p < 0.01$) with uniform flow rate, showed no differences in the arterial pressures during the perfusion ($p > 90$).

The weight increase varied from 41 to 55% in the different groups (Table III). No attempts to compare the groups statistically were made, because of little knowledge concerning the influence of varying flow rates on the weight increase.

In all groups the pressure decreased during the first 15–40 min of the perfusion (Table II). After 40 min the pressure increased slowly in nearly all groups, probably because of the increasing oedema which developed during the perfusion and caused a weight increase of about 50%.

Group 1 shows the arterial pressure in perfusions without addition of heparine and vasodilators to the perfusate medium, and without heparinization of the animals before removal of the kidneys. The pressure reaches the lowest value (40 mmHg) after 40 min, and the S.D of the means is relatively large (Table II).

Groups 2 and 3 show the arterial pressures when heparine was added to the perfusate (group 2) and when the animals where heparinized before removal of the kidney (group 3), but without use of vasodilators. Both groups are characterized by reaching the lowest values after 40 min, high minimum levels (68–6 mmHg) and great S D.s of the means during the whole period (Table II). The pressures are higher in group 2 compared to group 3 but only significant at the start of the perfusion ($0.025 < p < 0.05$). Compared to group 1 higher values were found after 40 min perfusion ($p < 0.01$) in both groups, but at the start of the perfusion a significantly lower pressure was found in group 3 ($0.025 < p < 0.05$).

Group 4 shows the arterial pressure when the concentration of papaverine in the perfusate medium is 1 mg%. It will be seen that the pressure during the first 40 min is lower than that of group 1 and that this is the case especially during the first 10 min of the perfusion, where the difference is significant ($p < 0.01$). The lowest value (42 mmHg) was obtained after 30 min. The standard deviations of the means are (just as in group 1) relatively large in the beginning, but after 15 min essentially less than in group 1. No significant differences between the flows were found in the first four groups.

Table III. *The means of the measured values concerning flow temperature and weight increase in ten groups of rat kidney perfusions. The temperature is shown after 10 minutes perfusion. The S D s are stated*

Group no.	Flow (µl/g/min)	Temp. (°C)	Weight increase (%)
1	562±43	8.5±1.3	55±20
2	573±70	8.8±1.2	47±7
3	570±51	9.4±1.0	41±11
4	558±70	8.1±0.6	42±9
5	685±37	9.6±0.8	45±6
6	655±55	9.1±0.7	52±16
7	676±76	9.4±0.9	48±14
8	771±88	9.1±0.9	50±8
9	624±66	9.3±1.1	41±11
10	758±57	9.4±0.7	41±12

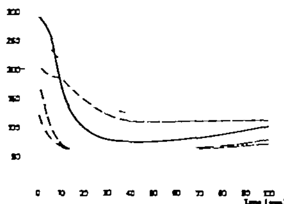


Fig. — Changes in the vascular resistance during hypothermic perfusion of rat kidneys. Group 1 (control group), —. Group 2 (heparine in the perfusate), -- Group 3 (heparinization before removal), - - Group 5 (5 mg% papaverine in the perfusate), --- Group 7 (heparinization before removal +5 mg% papaverine to the perfusate), - -

Groups 5 6 and 7 show th. arterial pressures when the concentrations of papaverine are 5 mg% No significant difference between the flows found in these groups, but compared with the first four groups the flows were found to be significantly higher ($p<0.01$). All the groups reached their lowest pressure values (40 mmHg) after about 20 min perfusion, and hereafter the curves are nearly identical. Group 5, in which only papaverine was added to the perfusate medium, and group 6 in which also heparine was added, show nearly identical curves during the whole period. Group 7 in which the animals were heparinized before removal of the kidney differs from the previous two groups, having a lower arterial pressure in the first 10-15 min of th. perfusion. The difference was significant after 1 and 5 min ($0.01<p<0.02$). A comparison between the use of 1 and 5 mg% papaverine shows a faster decrease in the pressure when 5 mg% papaverine was used (groups 4 and 5 $0.01<p<0.02$ after 10 min).

Group 8 shows the arterial pressure when the concentration of papaverine is 40 mg% in the perfusate medium and the animals are heparinized before removal of the kidney The pressure was found to be higher during the whole period when compared to group 7 This was probably due to a higher average flow in group 8 (about 14% $p<0.01$) Otherwise, the group behaved very much like the other groups in which 5 mg% papaverine was used, reaching the lowest pressure after 20 min and showing only small S.D.s of the means.

Groups 9 and 10 show the arterial pressures when the concentrations of lidocaine were 50 and 100 mg% The animals in both groups were heparinized before removal of the kidneys. Five min after start, group 9 shows a significantly lower pressure compared to group 1 ($p<0.01$), and the lowest value was obtained after 30 min (45 mmHg). The flow was about 9% higher in group 9 compared to group 1 ($0.025<p<0.05$). Group 10 reached the minimum level after 20 min and behaves like the groups in which 5 and 40 mg% papaverine was used. The higher flow found in group 10 compared to the groups using 5 mg% papaverine (about 12% $p<0.01$) is possibly responsible for the slightly higher minimum value in group 10 (47 mmHg)

DISCUSSION

The experiments show that the arterial pressure is higher at the beginning of the hypothermic perfusion than later on in the perfusion. Using a flow of about 0.5 ml/g/min the arterial pressure was about four times higher at the start than after 40 min perfusion when no vasodilators were added to the perfusate medium (group 1).

For purposes of comparison it is important to consider not only pressure but also flow since variations in flow may cause changes in the arterial pressure, which are unrelated to the addition of vasodilators or constrictors. A uniform flow rate (0.5 mg/g/min) was aimed at in all experiments, but was not constantly achieved (Table III). Although the relation between flow and pressure at various times of the perfusion has not been systematically investigated under these experimental conditions, it was observed that increase and decrease in the flow rate was followed by increase and decrease in the arterial pressure. When this factor is taken into consideration, the alleged increases and decreases in vascular resistance with addition of different drugs are in all probability even more significant than a mere comparison of arterial pressures has revealed.

A calculation of vascular resistance from flow and perfusion pressure can be made from th.

conventional formula. vascular resistance = pressure/flow The calculated vascular resistance for the control group and the four main experimental situations (without correction for possible changes due to variations in the flow rates) is illustrated in Fig. 2, and apparently permits the following conclusions.

Concentrations of 5 mg% papaverine and 100 mg% lidocaine in the perfusate medium halved the time for the vascular resistance to decrease to the minimum level. At the same time a much smaller dispersion of the pressures was found (Table II).

Addition of heparine to the perfusate medium increased the vascular resistance. This was especially pronounced after 40 min perfusion, when a significantly higher pressure was found compared to a group without any addition to the perfusate medium ($p < 0.01$).

Heparinization of the animals before removal of the kidneys caused a similar increase in the vascular resistance during most of the perfusion period, but the start pressure was lower than that of groups 1 and 2 ($0.025 < p < 0.05$). Using 5 mg% papaverine in the perfusate medium the start pressure was significantly lower in group 7 in which the animals were heparinized before removal of the kidney compared to group 5 in which no heparine was used ($p < 0.01$). This indicates that anticoagulation of the donor before removal of the kidney will facilitate the start of the perfusion, on the assumption that the time of warm ischaemia is of the same order of magnitude as in our experiments (7 min).

The increased vascular resistance due to heparine could be neutralized by means of papaverine and lidocaine, and the combination of heparinization of the animals before removal of the kidney and the use of 5 mg% papaverine or 100 mg% lidocaine revealed the best results, as judged by a low start pressure and fast decrease in the pressure.

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THE EFFECTS OF PARENTERAL CYSTEINE TREATMENT IN OLD PATIENTS

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Abstract. Several authors report that the tissue concentrations of -S-S- and -SH alter with age towards decreasing ratio -SH/-S-S- and it is claimed that this would cause detrimental functional changes. Furthermore, parenteral treatment of aged animals and humans with cysteine is claimed to increase the ratio and better the clinical condition. In ten patients, 67–90 years of age, in a stable condition and serving as their own controls, a battery of about 50 clinical chemical tests was used before, during, between and after two or three treatment periods with cysteine and folic acid intramuscularly. The only significant changes observed were in the serum concentrations of aspartate aminotransferase (GOT) and ammonium, and in the urinary excretion pattern of 17-ketosteroids. No alterations were found in the blood concentrations of oxidized and reduced glutathione. The clinical state was not ameliorated. The discrepancy between these and earlier results is discussed.

All living organisms undergo chemical, cellular and, consequently, functional changes with age. Among the numerous components which alter are those containing thiol groups (–SH). Most investigations have been concerned with the amino acid cysteine

$$HS \cdot CH_2 \cdot CH(NH_2) \cdot COOH \quad (CySH)$$

and the tripeptide (reduced) glutathione

$$HOOC \cdot CH(NH_2) \cdot CH_2 \cdot CH_2 \cdot CO-$$
$$NH \cdot CH(CH_2 \cdot SH) \cdot CO-$$
$$NH \cdot CH_2 \cdot COOH \quad (GSH).$$

These "reduced" forms are easily oxidized in vivo into the disulfidic compounds (–S–S–) cystine (CySSCy) and "oxidized" glutathione (GSSG) respectively.

Several investigators have reported that in animals and humans the concentrations of –S–S– compounds in tissues increase with age, whereas the concentrations of –SH groups decrease (17). It has been postulated that this shift is a major cause of the changes in the concentration and renewal rate of proteins and amino acids as well as in the activity of several enzymes, many of which are dependent on –SH groups. Parenteral treatment of aged organisms with cysteine was reported to alter the values of several biochemical parameters towards those found in young organisms (17, 18), and Harman (7) reported significant prolongation of the half-survival time of mice receiving cysteine or other antioxidants in the diet. Given the opportunity it was decided to investigate the effects of cysteine administration in patients serving as their own controls. A number of examinations were made to reveal whether functional changes occurred in different organs.

MATERIAL

Five women aged 80–90 years and five men aged 67–89 years consented to cooperate in the experiment. They were selected because: 1) adequate blood sampling and urine collection could be expected, 2) they were rather mobile and clinically stable, and 3) they required minimal medication. The patients did not receive modern psychopharmaca, hormones or iodine.

Most of the patients undoubtedly had universal atherosclerosis and myocardial degeneration as revealed by electrocardiography; none were markedly decompensated. All had normal molar concentration of cholesterols (total) in serum: < 7.0 mmol/l (= 2.69 g/l). Roentgenographically no recent changes were found in the lungs. Resting blood pressures (mean of two determinations) were below 200/125 mmHg; the parameter (diastolic pressure + 1/3(systolic–diastolic pressure)) was below 150 mmHg. Their body masses (46–78 kg) were within 0.85 to 1.15 × ideal mass (according to height), except in one case (1.39 × ideal). The molar concentrations of haemoglobin(Fe) in blood were normal, 8.4–9.1 mmol/l (= 135–147 g/l). The molar concentrations of creatininium(total)

in serum were normal, 0.07–0.11 mmol/l (= 8–12 mg/l), and examination of urinary sediment showed no overt infection.

The liver function tests were within the 0.95-interval for normals in most cases or just above the upper limit in single cases. The preliminary tests comprised thymol reaction of serum, enzyme concentrations in serum of alkaline phosphatase, aspartate aminotransferase (GO transaminase), lactate dehydrogenase, as well as sulfobromphthalein loading.

No signs of endocrine disorders, cancer or lues were found, none of the patients had undergone gastric resection.

METHODS

The ten patients entered the experiment at different times during the period June 27 1966, to Jan. 9 1967 and the last blood sample was taken on Sept. 20, 1967 The patients submitted to the following schedule.

A pre-treatment period of 14 days (symbolized below by *N0*) was followed by a first treatment period of 40 days (*T1*), a non-treatment period of 40 days (*N1*), second treatment period of 40 days (*T2*), and a final non-treatment period of 40 days (*N2*).

The treatment consisted of deep intramuscular injections every second day of 5.0 ml of "Folevistene-Oertr" containing cysteine chlorhydrate 250 mg and folic acid 5 mg.

Four of the patients, two women and two men, underwent further 40 plus 40 days of treatment (*T3*) and non-treatment (*N3*), respectively receiving double dosage during *T3*. (Ampoules for the entire experiment supplied by Dr S. Oertr.)

Blood sampling. The detailed technique has been described earlier (4)

4-hour urine as collected without preservative. The efficiency of collection was checked by measurement of the mean mole rate of creatininium excretion, which was found rather constant in each patient. The mean volume rate of urine excretion was seldom below 500 ml/d.

The laboratory technicians were not aware of whether a sample came from patient during treatment or non-treatment period. (Analyses for blood concentration of GSH and GSSG performed by Medicinsk Laboratorium, Copenhagen.)

Tests were mostly performed according to six different schedules (symbolized in the following by the numbers):

1. Before the start of the experiment.
2. At the end of each period (N0, N and T).
3. Twice during N0 and once at the end of each T and N
4. Twice during N0 and three times during each T and N (4 11 and 39 days after the start of each period).
5. Three times during N0 (0, 7 and 11 days after the start) and three times during each T and N (4 11 and 39 days after starting).
6. T ice daily

The tests performed are listed below and the schedules are indicated.

In spite of the original clinical assessment one female patient became too incontinent to allow adequate collection of urine; consequently all results of hormone analyses in urine from this patient were discarded.

Statistical methods

It has been presumed that each result of a single measurement of given quantity is the sum of three independent elements: contribution from the particular patient, contribution from the treatment, and final contribution of stochastic variation, independent of that in other results in the same period, and normally distributed around mean equal to zero. The ariance of the last element was found to be the same from patient to patient and, for given patient, from period to period, therefore common estimate of the variance of this element (s_p^2) was calculated from the sum of the individual period variances.

For each patient and each measured type of quantity the difference between the average f results during treatment (*T1*+*T2*, and sometimes +*T3*) and the average of results during non-treatment (*N0*+*N1*+*N2*, and sometimes +*N3*) was calculated (d_i). The weighted mean of differences from all ten patients (d) was determined according to $d = \Sigma f_i d_i / \Sigma f$ where the individual degrees of freedom f were calculated from the individual number of determinations during treatment (n_{tr}) and non-treatment ($_{tr}$) according to the equation $1/f = 1/ \quad + 1/$ The significance of any deviation of d from zero may be t-tested using the variance $s_p^2/\Sigma f$

For the adrenocortical hormones in urine the observations had to be transformed into their logarithms in order to obtain normal distribution. Furthermore, a strong correlation between the results of the fi frac tions were found. Consequently the question of significance was further explored by vector analysis using variance matrix and a χ^2-test. (Statistical calculations made by H. Engberg-Pedersen.)

RESULTS

The concentrations in blood of the two free "glutathione forms" GSH and GSSG should be of special interest in relation to the treatment with the –SH containing cysteine. The mean values during non-treatment and during th two intensities of treatment are shown in Table I. As might be expected from these means, th original data showed no significant differences at the 0.05 level when treatment periods were compared with non-treatment periods.

Among the several liver functions the enzyme concentration of aspartate aminotransferase (GOT) in serum (16) showed an average increase during therapy of 1.6 U/l. This is significant ($p \approx 0.002$), but the difference is small in relation to the overall mean value of 18.3 U/l. The molar concentration of ammonium in serum (3 9)

showed an average increase of 7.6 μmol/l during therapy. This difference is significant ($p \approx 0.004$) and considerable in relation to the overall mean, 38.6 μmol/l, but the abnormal range is not reached.

For most parameters measured no significant changes could be detected during therapy. (In the following the number following the component name and a colon refers to the type of schedule, cf. Methods, whereas the reference number or remark is in parentheses.) Thus the *lipid* and *glucose metabolism* was normal as evidenced by the concentrations in serum (S) or plasma (P) of

S–Cholesterols (total): 3 (modified Liebermann–Burchard),
S–Cholesterol (non-esterified): 3 (modified Liebermann–Burchard)
S–Fatty acid (esterified): 3 (4),
S–Triglycerides: 3 (14),
S–Phospholipid (P): 3 (4),
fasting P–D-Glucose: 3 (28), and response after D-glucose loading: 2 (28)

Also the *cardiovascular, thyroid* and *renal functions* seemed unchanged by therapy as seen by the patients

Pulse rate: 6 (standard procedure),
Resting blood pressure: 3 (standard procedure),
Electrocardiogram with standard limb leads and six unipolar precordial leads: 3 (standard procedure)

and by the molar concentrations in serum (S) of
S–Iodine (I, protein-bound): 3 (Technicon® Autoanalyzer),
S–Triiodothyronine (total): 3 (29),
S–Creatininium: 3 (8),

as well as by the urinary (U) concentrations of
U–Protein: 3 (Labstix®)
U–D-Glucose: 3 (Labstix®)
U–Blood: 3 (Labstix®)

the 24-hour urinary (dU) volume and its amount of substance of
dU–Creatininium: 3 (30)

Furthermore the *hematological parameters* in the form of blood (B) concentrations of
B–Hemoglobin: 3 (12)
B–Erythrocytes: 3 (Coulter Counter® model D),
B–Leukocytes: 3 (Coulter Counter® model D),
B–Sedimentation reaction: 3 (4),

were all unchanged, as were those of *coagulation parameters*

P–Fibrinogen: 3 (4),
P–Coagulation factors II+VII+X: 3 (4),
P–Thrombin generation reaction: 3 (4)
B–Platelets: 3 (4)
B–Platelets (adhering): 3 (4).

Additionally
X-ray of thorax: 2 (large film),
Rectal temperature: 6
Body mass: 3

remained stable.

All other *function tests* remained unchanged, i.e. the concentrations of
S–Alanine aminotransferase (= GPT): 4 (4)
S–Lactate dehydrogenase: 4 (15),
S–Alkaline phosphatase: 4 (4),
S–Thymol reaction: 3 (4),
S–Carbamide (= urea): 3 (3),
S–Urate: 3 (26, 27),
S–Protein fractions: 3 (1, 2),

as well as the response in the concentration of S–Sulfobromphthalein to sulfobromphthalein loading of the patient: 1 (31).

Table I. *Molar concentrations in mmol/l of free "reduced" glutathione (GSH, M = 307.2) and free "oxidized" glutathione (GSSG, M = 612.4) in blood*

Means of individual means during treatment (T) and non-treatment (N) periods

	Ten pats.		Four pats.	
	N 0, 1, 2	T 1, 2	T 3	N 3
GSH	0.88	0.86	0.81	0.86
GSSG	0.078	0.079	0.085	0.070

Table II. *24-hour urinary 17-ketosteroid fractions*

Mean value during treatment (T) relative to mean value during non-treatment (N)

Quantity	T/N	p for T against N
Androsterone, mass (A-fraction)	0.92	> 0.05
Dehydroepiandrosterone, mass (DHA-fraction)	0.85	0.002
α-Etiocholanolone, mass (E-fraction)	0.82	< 0.001
U-fraction, mass	0.87	> 0.05
R-fraction, mass	0.95	> 0.05
Androsterone, mass/α-Etiocholanolone, mass (A/E ratio)	1.13	0.025–0.01

The *genito-adrenal function* was shown by the 24-hour urinary excretion of 17-ketosteroids (schedule 5 ref 6) of which the following were unchanged.

dU–Androsterone (= A-fraction),
dU–U fraction
dU–R-fraction,

whereas some were decreased

dU–D.hydro-piandrosterone (= DHA-fraction),
dU–α Etiocholanolone (= E-fraction)
dU–Androsterone + α Etiocholanolone (= A + E), and, consequently an increase was found in
dU–Androsterone/α-Etiocholanolone (= A/E ratio)

The relative changes in some genito-adrenal hormonal fractions (10) are shown in Table II. The total 24-hour excretion of 17 ketosteroids decreased, but no significant alterations could be detected in the androsterone and R-fractions. The dehydroepiandrosterone and α-etiocholanolone fractions decreased about one sixth, which proved significant. The androsterone/α-etiocholanolone ratio increased one eighth, which is also significant. The overall picture of correlated fractions was examined by vector analysis and the χ^2-test showed significance ($p \approx 0.002$), i.e. the excretion pattern is changed by cysteine medication.

Supplementary clinical observations

A few patients suffered short episodes of influenza-like fever seemingly without relation to the treatment. The parameters measured were not influenced to a significant degree

One patient developed a bronchopneumonia during *T1* and was rapidly cured by penicillin a few parameters at the end of *T1* were altered in this case without changing the overall picture. During *N1* the same patient had a short bout of clinical arthritis urica, treated with phenylbutazone and not influencing the parameters.

In one case with a history of duodenal ulcer melena occurred at the end of *T2*, and many values were changed they reverted to earlier levels in about 50 days and the general trend was not altered. The patient later underwent *T3* (double dosage) without mishap

DISCUSSION

A number of experiments have been reported showing changes with age in –SH containing or dependent compounds. Much of the literature is in Eastern European languages or in the form of short communications, but some first-hand sources are accessible.

In experiments on rats, rabbits and humans, varying small changes with age in the blood concentrations of "reduced" glutathione (GSH) and considerable significant ($p \leq 0.01$) increases in the "oxidized" glutathione concentrations (GSSG) have been found (23 24). In men, 20–30 years of age the GSSG mean mass concentration in blood was 3.5 mg/100 ml at 50–60 years 5.9 and at 70–90 years 6.8 (23)

Treatment of aged rats with cysteine caused a decrease in the mean concentration of GSSG in blood and an increase in GSH (24 25) cysteine + folic acid lowered the GSSG concentration more than cysteine alone (25). In fact, the treated old rats showed a significant ($p < 0.01$) fall to a mean value *below* that in young animals and a concomitant *fall* in GSH also to a sub-juvenile level.

In some of these reports and several others by Oeriu and co-workers (17 19 20, 21 22) the changes with age in the concentrations in blood or serum of water-soluble vitamins, amino acids and some enzymes were also registered. Furthermore they demonstrated effects of treating old animals and man with methionine cysteine or cysteine with one of several water-soluble vitamins of the B-group added. It was concluded that cysteine + folic acid gave results which resembled the state in young animals.

The design of the present experiment in aged humans allows each person to be his or her own control and individual differences in level or reactivity can thus be cancelled. In ten patients the intensity of treatment with cysteine + folic acid during the two first periods (*T1* *T2*) was that used by Oeriu et al. (19) and by Oeriu and Tighciu (23) in four patients the dose rate was doubled during a further period (*T3*)

The results showed that the lipid and glucose metabolism as well as the cardiovascular thyroid and renal functions seemed unchanged by cysteine medication as did the hematological and coagulation parameters. Most parameters reflecting liver function were not significantly altered. The reported decreases in serum concentrations of aminotransferases during treatment (19) could not be substantiated on the contrary we found

a slight increase in the serum concentration of aspartate aminotransferase (GOT). The materials are not fully comparable, however as Oeriu et al. (19) treated for longer periods and also gave "thiazolidinecarboxylic acid as an active principle *which by an enzymatic mechanism sets free the* SH group

The augmentation in the molar concentration of ammonium in serum during treatment (difference 7.6 μmol/l as against a mean of 38.6 μmol/l) seems not to have been reported before and could be a sign of altered liver function. The small increase in the concentration of aspartate aminotransferase might point in the same direction.

The only other changes registered during treatment were in the urinary excretion rate of 17-ketosteroid fractions (Table II). The single report on these parameters (18) showed an increase in the low level for androsterone in old men. In our material a non-significant decrease was seen in the androsterone fraction. The same was true for the male patients alone; the function of the testes, therefore, seems unaltered.

A small significant decrease in the dehydroepiandrosterone excretion rate could be explained by a change in the steroid metabolism of the liver (6), cf. the two altered parameters of liver function.

The significant decrease in α-etiocholanolone excretion rate and concomitant increase in androsterone/α-etiocholanolone ratio is characteristic *of hypothalamic disease and of hyperthyroidism* (11): it may be found also when the liver function is altered (6). The first cause, as a reversible result of treatment, seems unlikely: the second is not corroborated by the unchanged tests of thyroid function. Again, an altered liver function would explain the finding.

It was surprising that no significant changes could be detected in the concentrations of "oxidized" or "reduced" glutathione in blood. The concentration of GSSG was slightly higher during treatment with –SH groups than without (Table I). This contradicts the cited findings by Oeriu *and co-workers. Their human subjects received* the same standard dosage as ours, albeit for two plus two months with a non-treatment interval of 14 days (23). They did not assay the double dosage intensity used in four of our patients. There is no obvious explanation of this discrepancy apart from different methods of analysis, which also preclude a meaningful comparison of absolute values. The measurements of GSH and GSSG in blood notoriously have presented difficulties, but the enzymatic method (13 somewhat *modified) used in the present work is considered* reliably specific.

The paucity of changes during administration of cysteine found in the present investigation does not preclude that a higher dosage or a much longer period of treatment would have influenced some parameters in the manner reported by Oeriu and co-workers. That effects can be elicited has been shown in mice (5). A considerable increase in half-life was registered when their diet was augmented by small amounts of antioxidants (e.g. di-*ter*-butyl hydroxytoluene or 1,2-dihydro-6-ethoxy 2,2,4-trimethylquinoline). These compounds reduce the concentration of free radicals in the diet and in the body reinforcing the analogous effect of the naturally occurring –SH compounds and α-tocopherol. Presumably the antioxidants decrease the rate of deleterious changes in essential macro-molecular bindings, continuously produced by free radicals and postulated as a causative mechanism in ageing.

CONCLUSIONS

In spite of using a rather large battery of tests on ten old patients, serving as their own controls, it proved impossible to reproduce the reported *effects of a recommended dosage of cysteine* (+ folic acid) in old persons. Especially no significant changes were seen in the concentrations of "reduced" and "oxidized" glutathione in the blood.

Modest increases during treatment in the concentrations in plasma of ammonium and in serum of aspartate aminotransferase (GOT) might indicate an altered liver function. This in itself could explain the significant moderate shifts found in the urinary excretion rates of 17-ketosteroid fractions.

The treatment seemed to be without clinically detectable side effects.

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THE EFFECT OF PHLEBOTOMY THERAPY IN PORPHYRIA CUTANEA TARDA

Its Relation to the Phlebotomy-Induced Reduction of Iron Stores

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Abstract Porphyrin excretion and clinical signs have been studied in 21 patients with porphyria cutanea tarda treated by phlebotomy and in control series of 12 patients. Phlebotomy was performed until signs of depleted iron stores appeared. Clinical and biochemical remission occurred in every phlebotomy-treated patient. In 18 patients who were bled frequently (usually 0.5 l per week) and who were not given iron supplements in conjunction with phlebotomy the urinary porphyrin excretion decreased in all to level of 1 mg per day or less within / year Remission ensued in patients with iron overload as well as in patients with quantitatively normal iron stores and in patients abstaining from alcohol as ell as in patients with persistent abuse of alcohol. A decrease in porphyrin excretion comparable to that of phlebotomy-treated patients occurred in only two out of the 12 control patients. There was significant relationship between the period required until iron stores were depleted and until the urinary porphyrin excretion fell to 1 mg per day In one patient given iron supplement for 6 / months in conjunction with phlebotomy more blood had to be removed to obtain remission than in any other patient, and this patient had the longest treatment (16 months). Thus the results have shown that phlebotomy therapy is effective in porphyria cutanea tarda. It seems probable that the effect is mediated through the phlebotomy-induced reduction of iron stores.

Phlebotomy therapy of porphyria cutanea tarda (PCT) was introduced by Ippen in 1960 (11) and since then several reports on the effect of phlebotomy therapy have appeared. In some series this treatment was consistently associated with remission (7 13 20) but in others the results were less impressive (10 14, 36). The evaluation of any therapy in this disease may be difficult because remission may occur spontaneously or may ensue with abstinence from alcohol. Hence, in the present study which was aimed at assessing the effect of phlebotomy therapy a control series was included and care was taken to evaluate the alcohol habits before and after the start of therapy

Whether the effect of phlebotomy therapy is due to reduction of iron stores or to some other mechanism is not known. In order to study this question the effect of phlebotomy on porphyrin excretion was studied in relation to the depletion of iron stores. Some of the present results have been reported in a preliminary study (20).

MATERIAL AND METHODS

Thirty-one patients (24 men and 7 women) are included in the study The age distribution at the onset of skin symptoms and at the start of the observation (or treatment) period is shown in Table I, hich also shows the duration of the disease at the start of the treatment (or observation) period. Two patients are twins (nos. *14* and 31) and have been reported previously by Waldenström and Haeger-Aronsen (35). Two patients are brother (no. 3) and sister (no. 28), and one female patient (no. 30) has brother with clinically latent PCT ho is not included in this study Another patient (no. 9) has tw brothers with latent porphyria cutanea tarda. In the rest of the patients there was no family history of porphyria.

All had typical biochemical and clinical signs of PCT Skin fragility on skin areas exposed to sun was constant symptom. Most patients also had blisters now and then. Hyperpigmentation was judged to be present in two-thirds of the patients. Gross sclerodermal changes ere present in one patient (no. 23). Cosmetically disturbing hypertrichosis was present in four of the women.

All had gross uroporphyrinuria. Coproporphyrin excretion in urine was in most cases slightly increased. Excretion of porphyrin precursors in urine was normal. Faecal coproporphyrin excretion was slightly to moder-

Table I. *Alcohol consumption during the years immediately before the onset of skin symptoms and the last half year before the observation (or treatment) period. The table also shows the estimated alcohol consumption during the observation (or treatment) period*

Alcohol consumption was calculated in litres of 40% (v/v) ethyl alcohol per month, and graded as: heavy (more than 6 l), large (3.1–6.0), moderate (1.1–3.0), small (0.4–1.0), minimal (0.1–0.3) and insignificant (less than 0.1 l/month) consumption

Pat. no.	Sex	Born	Age[b]	Alcohol consumption	Previous history	Start (age)	Alcohol consumption: Before	During	Duration (y)
						Observation period			
1 (12)	♂	1899	67	Heavy	Lues 1920 Epileptic fits 1960	67	Heavy	Insign.	[illegible]
2 (16)	♂	1896	72	Large	Lues 1928	72	Large	Small	[illegible]
3	♂	1912	46	Heavy	Diabetes 1961	56	Heavy	Insign.	10
4	♂	1905	63	Large	Myocardial infarction 1958	63	Large	Small	[illegible]
5 (19)	♂	1903	64	Moderate		64	Moderate	Insign.	[illegible]
6 (21)	♂	1920	47	Moderate		47	Moderate	Small-moderate	[illegible]
7 (18)	♂	1923	44	Moderate		44	Moderate	Small-moderate	[illegible]
8 (8)	♂	1923	40	Large	Infectious hepatitis 1950	41	Large	Large	2
9 (1)	♂	1910	57	Heavy		57	Sporadic	Sporadic	0
10	♀	1899	68	Minimal		69	Minimal	Minimal	1
11 (20)	♂	1904	63	Minimal	Diabetes 1963 Parkinson's disease 1963	63	Minimal	Minimal	[illegible]
12	♂	1904	61	Insign.		64	Insign.	Insign.	3
						Venesection therapy			
13 (14)	♂	1912	53	Large		53	Large	Large	[illegible]
14 (6)	♂	1917	35	Large		49	Large	Large	14
15 (13)	♂	1912	50	Heavy		55	Heavy	Heavy	5
16 (17)	♂	1909	55	Heavy		59	Heavy	Heavy	4
17 (2)	♂	1922	44	Moderate		45	Moderate	Moderate	[illegible]
18 (11)	♂	1917	48	Minimal	Diabetes 1962	50	Minimal	Minimal	1[illegible]
19 (22)	♀	1898	69	Insign.	Epileptic fits 1960	69	Insign.	Insign.	[illegible]
20 (10)	♂	1907	50	Heavy	Myocardial infarction 1967	60	Insign.	Insign.	10
9 (1)	♂	1910	57	Heavy		58	Insign.	Insign.	1[illegible]
21 (9)	♂	1910	55	Moderate		57	Moderate	Moderate	2
22 (3)	♂	1902	62	Moderate	Diabetes 1960 Myocardial infarction 1963	63	Moderate	Moderate	1
23 (26)	♀	1902	59	Insign.	Epileptic fits 1922	64	Insign.	Insign.	5
24 (24)	♀	1916	52	Large		52	Large	Insign.	[illegible]
25 (27)	♀	1943	23	Large		23	Large	Small	[illegible]
8 (8)	♂	1923	40	Large	Infectious hepatitis 1950	42	Large	Small	2[illegible]
26 (5)	♂	1919	43	Moderate		47	Moderate	Small	4[illegible]
27 (4)	♂	1892	73	Moderate	Lues 1918 Myocardial infarction 1939	74	Moderate	Small	1
28 (25)	♀	1925	38	Small		40	Small	Insign.	2[illegible]
29 (15)	♂	1917	48	Moderate		49	Small	Insign.	1
30 (23)	♀	1927	39	Minimal		41	Minimal	Insign.	2
31 (7)	♂	1917	42	Heavy		50	Heavy	Large	4

Figures in brackets refer to patient numbers used in a previous report on storage iron in PCT (23).
[b] Age at the onset of skin symptoms.

ately increased in most patients, while protoporphyrin faecal excretion was increased less often.

The amount of alcohol consumed was estimated by repeated interviews with the patients and in most instances with relatives too. As shown in Table I, excessive alcohol consumption was common.

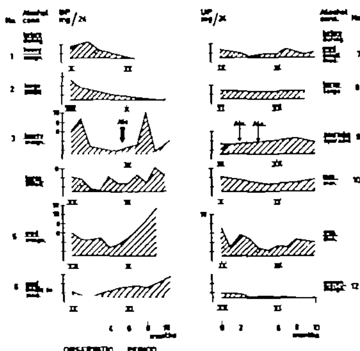


Fig. 1. Urinary uroporphyrin excretion in 12 patients with porphyria cutanea tarda during observation periods of about 1 year during which no treatment was given. Hatched areas represent uroporphyrin excretion exceeding 1 mg/day. Roman numerals represent ordinal numbers of month.

All but four (nos. 3, 4, 10, 12) of the present patients were included in an earlier report on iron storage in PCT (23), and the figures within brackets in Table I refer to the patient numbers used in that report.

The control group (Table I, nos. 1 to 12) includes 12 patients in whom clinical signs and urinary porphyrin excretion were observed for periods of at least 1 year during which no treatment was given. At the start of the observation period their mean age was 59 years with a range of 41 to 72 years, and the median duration of their disease was 1 year with a range of 1 to 10 years. Two patients (nos. 8 and 9) were later phlebotomized and are also included in the phlebotomy-treated group. Eight in this group had had a regular moderate to heavy consumption of alcoholic beverages for many years. They were informed that alcohol probably has an unfavourable influence on the disease and were advised to avoid alcohol. They were asked to register their consumption of alcohol if they chose not to abstain totally. In most patients (in all but no. 10) information as to alcohol consumption during the observation period could be obtained from relatives. The estimated alcohol consumption before and during the observation period is given in Table I.

In the control group one patient (no. 1) was on anticonvulsive treatment (diphenyl hydantoin) because of posttraumatic epileptic fits, and two patients (nos. 3 and 11) with diabetes were treated with tolbutamide. These medications were kept unchanged during the observation period. One patient (no 10) had ingested iron preparations regularly since she was a teenager. The iron consumption was stopped at the start of the period of observation.

The urinary porphyrin excretion was determined monthly if possible. Faecal excretion of porphyrins (coproporphyrin and protoporphyrin), serum bilirubin, SGOT, SGPT and bromsulphalein retention (BSPR) were determined initially and at the end of the observation period.

The phlebotomy-treated group (Table I, nos. 13 to 31 and nos. 8 and 9). The effect of phlebotomy was studied in 21 patients. At the start of phlebotomy therapy their mean age was 52 years with a range of 23 to 74 years and the median duration of their disease was 2 years with a range of 1 to 14 years.

The patients were asked to register their alcohol consumption during treatment. In all but two (nos. 15 and 16) information as to alcohol consumption during the treatment and the following observation period could be obtained from relatives or other persons close to the patients.

In nine patients the alcohol consumption was judged to be more or less decreased during treatment as compared to that before treatment (nos. 24 to 31 and no. 8). Two of these nine patients had been on sex hormones, viz. norethandrone and mestranol, as contraceptive agents (no. 25) and estrogens because of climacteric symptoms

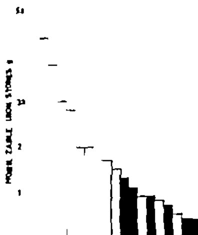


Fig. 2. Mobilizable iron stores (storage iron available for hemoglobin synthesis at repeated phlebotomy) in 18 patients with porphyria cutanea tarda. Black bars represent women.

(no. 24). These medications were stopped some months before phlebotomy therapy

In 12 patients the alcohol consumption was estimated to be essentially unchanged during the treatment period and the following observation period as compared to that before treatment (nos. 13 to .3 and 9). T of these (*nos. 19 and 23*) *were on anticonvulsive treatment* (diphenyl hydantoin and phenobarbital), and two had diabetes mellitus and were treated with tolbutamide (no. 18) or chlorpropamide (no. 22). These medications were kept unchanged during the phlebotomy therapy

Two patients ere given iron in conjunction with the phlebotomy therapy In one (no. 22) 300 mg of iron daily (as ferrous sulphate) was given orally for 6 / months. In the other (no. 21) 2 700 mg of iron (as iron dextrin) as given intravenously during a period of ten eeks.

The effect of the phlebotomy treatment was studied by evaluation of clinical symptoms and by regular determination of urinary porphyrin excretion. In part of the series porphyrin fluorescence was studied in cytological aspiration liver biopsy specimens. Determination of faecal porphyrins (coproporphyrin and protoporphyrin) and liver function tests were made before treatment and after remission had occurred.

The phlebotomy treatment usually consisted in removal of 0.4–0.5 l of blood at weekly intervals until signs of depleted iron stores appeared. At each phlebotomy hemoglobin was determined. Serum iron and total iron binding capacity (TIBC) were determined at each or every other phlebotomy When the hemoglobin concentration had fallen to 10.5 g/100 ml or below this level the phlebotomies were stopped. When this level was reached the hemoglobin concentration and serum iron were determined weekly for three to four eeks. If during this period the hemoglobin level remained unchanged and the serum iron was persistently low it was concluded that no storage iron available for hemoglobin synthesis was present. If the hemoglobin concentration tended to rise within this period, more than could be accounted for by an absorption of iron from food of 3–5 mg/day the phlebotomies were repeated until signs of depleted iron stores according to the above criteria were achieved. One patient (no. 15) *moved to another town, and phlebotomy was discontinued* before signs of iron depletion appeared. In some patients the circumstances did not allow the performance of weekly phlebotomies without temporary stop and in one (no. 23) the phlebotomies could be made only once or twice monthly The number and frequency of phlebotomies for each patient re shown in Fig. 3

The desferrioxamine-induced urinary iron excretion was determined befor and after phlebotomy The determination after phlebotomy was made t a time when the curtailed hemoglobin production indicated that iron stores were depleted. In most patients also sternal marrow was studied before and after therapy with respect to reticular hemosiderin nd sideroblasts.

Staining and examination of reticular hemosiderin and sideroblasts in bone marrow smears were performed as described by Hansen and Weinfeld (9). Liver function tests and the methods used for the determination of hemoglobin concentration, serum iron, total iron binding capacity (TIBC), desferrioxamine (DF)-induced urinary iron excretion, and mobilizable iron stores are the same as described in previous reports (19 21 23). Urinary uroporphyrin excretion was determined according to the principle of Svelnsson et al. (33) as described by Askevold (1). Recently the method elaborated by Dresel et al. (5), and described by Rimington (25), has been adopted for uroporphyrin determination in urines containing small amounts of porphyrins. Other methods used for the analysis of porphyrins and porphyrin precursors are the same as described in an earlier report (23). The technique used for the study of porphyrin fluorescence in cytological aspiration liver biopsy specimens, as well as the principles for the grading of the hepatic fluorescence, have also been described earlier (18).

RESULTS

Control patients (Fig. 1 Tables II III and IV)

In general, skin symptoms improved in the winter but usually skin fragility and ulcers did not vanish completely even in the dark season. In three patients (nos. 1 2 and 7) however the skin healed within two to three months and skin symptoms did not reappear within observation periods of 10 to 18 months. In two of these patients (nos. 1 and), who virtually had stopped their alcohol consumption the urinary uroporphyrin excretion diminished to 1 mg/day or below this level after seven (no. 1) and nine (no.) months (Fig. 1). One of the latter patients (no 1) was observed for another eight months. During this period he resumed alcohol consumption and

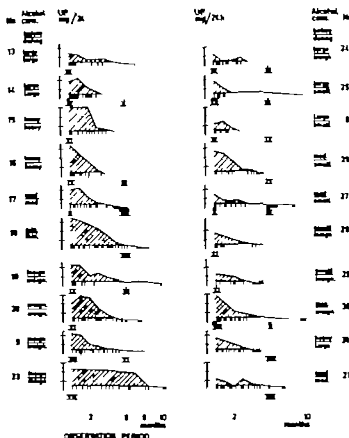


Fig. 3. Urinary uroporphyrin excretion during periods of 10 months after the start of phlebotomy treatment in 20 patients. The vertical bars at the base line of the chart of each patient represent venesections of 0.4 to 0.5 l. Other symbols as in Fig. 1.

Table II. *Porphyrin excretion initially and after an observation period of approximately ½ year in the control group*

ALA = delta-aminolevulinic acid. PBG = porphobilinogen. UP = uroporphyrin. CP = coproporphyrin. PP = protoporphyrin

			Urine				Faeces			
			UP (μg/24 h)		CP (μg/24 h)		CP (μg/g dry wt)		PP (μg/g dry wt)	
Pat. no.	ALA (mg/24 h)	PBG (mg/24 h)	Start	End	Start	End	Start	End	Start	End
1	4.2	1.2	3 400	400	240	100	52	14	22	8
2	3.1	0.6	5 400	1 000	250	90	55	40	20	30
3	4.5	1.9	6 100	4 000	450	450	70	7	106	13
4	5.0	0.8	4 100	4 400	290	190		47		82
5	0.3	1.3	5 800	11 600	240	480	40	60	17	28
6	1.3	1.3	2 100	3 600	300	160	23	67	23	101
7	0.2	0.4	2 600	2 300	170	200	21	31	12	24
8	3.0	0.7	2 800	2 700	70	360	28		7	
9	0.5	0.6	3 400	3 700	130	200	105		74	
10	3.2	1.3	4 200	3 600	370	270	15	27	13	24
11	0.3	0.7	6 900	4 000	280	270	12	64	25	44
12	2.3	0	1 900	1 000	140	90		7		9
Upper limit of normal			200		150		10		40	

Table III. *Liver function tests at the start and at the end of the observation period in control patients*

Upper limit of normal	SGOT (U.) 40		SGPT (U.) 40		Bilirubin (mg/100 ml) 1.1		BSPR ( ) 5	
Pat. no.	Start	End	Start	End	Start	End	Start	End
1	115	274[a]	162	95[a]	0.7	1.2[a]	32	23[a]
2	81	40	108	50	0.6	0.5	14	9
3	135	85	150	95	0.4	0.5	7	5
4	31	35	61	55	0.5	0.5	6	16
5	90	96	113	163	0.4	0.8	21	16
6	18	40	65	90	0.5	0.7	3	5
7	40	20	70	20	0.6	0.7	20	13
8	110	116	135	117	1.1	1.1		34
9	32	35	34	50	0.3	0.3	10	26
10	30	32	37	40	0.4	0.7	4	4
11	73	46	114	75	0.5	0.3	6	16
1	46	39	80	79	0.7	0.7	14	17

Tests at a time when the disease was complicated by hepatocellular carcinoma.

consumed about $1\,^1/_2$ l of hard liquor per month, but urinary porphyrin excretion remained at a low level (about 0.3 mg/day). However he developed ascites and a hepatocellular carcinoma was diagnosed. In patient 3 too, porphyrin excretion diminished considerably during total abstinence from alcohol. After a period of about 5 months he had a debauch and consumed $^3/$ l of distilled spirits daily for two weeks. He was then admitted to a mental hospital. In spite of no further alcohol consumption the uroporphyrin excretion during the following months increased to above 10 mg/day Later it decreased again.

In patient 4 the uroporphyrin excretion diminished initially when he abstained totally from alcohol. Later he resumed a small alcohol consumption (single glasses of beer and some time per week small quantities of distilled spirits) and this coincided with a higher uroporphyrin excretion. However during the last four months of the observation period he again abstained totally from alcohol but the uroporphyrin excretion remained essentially unchanged. In one patient (no. 5) who before the observation period consumed about two litres of hard liquor per month, porphyrin excretion did not decrease in spite of abstinence from alcohol. Patients 6 and 7 abstained from alcohol completely during the first months of observation, but later they resumed an irregular moderate alcohol consumption. A tendency to decreased porphyrin excretion existed during the period while they abstained from alcohol. In patients with unchanged alcohol consumption uroporphyrin excretion was essentially unchanged. In one patient (no. 11) the urinary porphyrin excretion showed large variations, however Faecal coproporphyrin and protoporphyrin as well as urinary coproporphyrin levels decreased in single control patients but remained essentially unchanged in most (Table II) As shown in Table III liver function tests of the group were essentially unchanged. At the start of the observation period all but two had increased SGOT and/or SGPT levels. Also at the end of the observation period all but two had increased SGOT and/or SGPT levels. Considerably decreased transaminase concentrations at the end of the observation period were, however present in three patients (nos. 2, 3 and 7). The BSPR was essentially unchanged in most control patients.

Results of histochemical estimation of iron in bone marrow films and the desferrioxamine test performed at the start of the observation period are shown in Table IV

Phlebotomy-treated patients (Figs. 2 to 7 Tables IV to VIII)

In general weekly phlebotomies of 0.4 to 0.5 l did not produce overt anemia until signs of depleted iron stores were present. In a few patients, however the phlebotomy treatment was discontinued because of anemia, and later resumed because the rate of the hemoglobin production indicated that the iron stores were not exhausted.

Table IV *Hemoglobin level, serum iron, desferrioxamine induced urinary iron excretion and stainable iron in bone marrow smears before and after phlebotomy therapy*

Pat. no.	Hb (g/100 ml)		Fe/s (μg/100 ml)		Stainable iron in bone marrow smears: Reticular (grade 0–4+)		Stainable iron in bone marrow smears: Sideroblasts (%)		DF-induced iron excretion: Total (mg/24 h)		DF-induced iron excretion: Per kg body wt (μg/24 h)	
Control patients												
1	14.0		235						1.10		16.9	
2	14.9		155		2+		80		1.32		15.5	
3	15.7		171		2+		65		2.40		30.1	
4	13.6		159		3+		52		1.37		14.0	
5	15.8		248		3+		84		1.91		23.6	
6	13.7		140		2+		79		1.17		13.0	
7	14.7		160		2+		63		1.62		16.2	
8	14.7		135									
9	13.1		177		4+		72		1.23		18.4	
10	13.6		239		2+		50		1.85		25.7	
11	14.5		200		3+		84		2.94		37.7	
12	16.8		124		2+		58		1.77		20.1	
Phlebotomy-treated patients												
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
13	14.6	9.7	133	42					0.73	0.40	9.4	5.1
14	15.8	10.2	189	28	3+		81		2.21	0.44	28.3	5.6
15	12.9	12.1	204	69					1.76		25.1	
16	13.8	10.0	145	34	2+	0	90	0	1.05	0.31	14.8	4.4
17	14.3	9.6	166	52	3+	0	77	0	1.07	0.54	13.0	6.6
18	14.2	10.3	300	58	2+		80		4.33	0.56	61.9	8.0
19	13.3	9.6	129	32	1+	0	45	4	0.99	0.43	13.2	5.7
20	14.0	9.9	234	39	3+	0	78	0	4.64	0.52	57.3	6.4
9	13.4	10.4	210	51	3+	0	80	0	3.25	0.14	48.5	2.1
21	14.8	9.3	247	30	1+	0	76	0	0.64	0.43	8.0	5.4
22	15.4	10.7	207	33	2+	0	72	0	2.59	0.46	32.0	5.7
23	13.4	10.7	114	48	3+	0	69	0	0.71	0.36	11.5	5.8
24	13.3	9.4	138	44	2+	0	65	0	1.07	0.18	16.0	2.7
25	12.8	10.0	206	34	2+		69		0.67	0.24	15.2	5.5
8	15.0	10.4	210	34					1.42		23.3	
26	14.4	10.0	168	44	3+	0	83	0	1.30	0.37	17.8	5.1
27	14.6	10.1	129	59	2+	0	61	0	1.16	0.47	13.6	5.5
28	13.6	10.0	206	42		trace	64	10	1.89	0.42	28.6	6.4
29	14.5	10.2	259	26	3+		86		1.30		14.0	
30	13.5	9.5	158		2+		75		1.25		19.2	
31	16.7	9.1	156	19					2.28	0.50	28.5	6.3

The mean amount of blood removed in patients who were not given iron supplements during phlebotomy therapy was 6.8 l with a range of 2 to 14 l (Table V). The amount of iron mobilizable from iron stores (Fig. 2) was calculated in 18 patients (12 men and 6 women) and ranged from 0.4 to 4.4 g with an average value of 1.7 ± 0.3 g. In the 12 men the range was 0.5 to 4.4 with a mean of 2.0 ± 0.4 g, and in the six women the range was 0.4 to 1.7 and the mean 0.9 ± 0.2 g. The average desferrioxamine-induced urinary iron excretion (Table IV) before phlebotomy therapy (23.8 ± 3.3 μg/kg body wt) was not different from that of the control porphyrics (21.0 ± [illegible] μg/kg) but was significantly higher than that of 26 normal men (10.1 ± 0.4 with a range of 6.9 to 14.9 μg/kg). Most patients also had rich amounts of reticular iron in bone marrow smears. High serum iron and sideroblast values were also common. The average DF-induced iron excretion value after therapy in 17 patients was 5.4 ± 0.3 μg/kg with a range of 2.1 to 8.0. This mean value was considerably lower ($p < 0.001$) than that of 26 normal men (10.1 ± 0.4), and all but one of the treated porphyrics had values below the normal range. The mean value was also significantly lower ($p < 0.01$) than that of 25 men who had donated

Table V *Volume of removed blood, amount of iron removed from iron stores, time required until phlebotomy caused depletion of mobilizable iron stores and period after start of treatment with a urinary porphyrin excretion above 1 mg*

Pat. no	Blood removed (l)	Iron removed from stores (g)	Period required until depletion of iron stores (mo.)	Period with urinary UP excretion above 1 mg/day (mo.)
13	4	0.5	2[illegible]	4[illegible]
14	8	2.0	4[illegible]	3[illegible]
15	6			4[illegible]
16	5	1 1	2[illegible]	3[illegible]
17	6	1.5	4[illegible]	3[illegible]
18	14	4.4	7[illegible]	6
19	2[illegible]	0.4	2	5[illegible]
20	13[illegible]	3.8	6[illegible]	4[illegible]
9	10[illegible]	3.0	6	4[illegible]
21	11[illegible]		5[illegible]	4[illegible]
22	22[illegible]		15[illegible]	16
23	7[illegible]	1 7	9[illegible]	8[illegible]
24	4[illegible]	0.9	3[illegible]	3[illegible]
23	2	0.4	1[illegible]	2
8	4	0.9	2	2[illegible]
26	8	1 7	6	4
27	5	0.8	3	3[illegible]
28	4[illegible]	0.7	2[illegible]	3[illegible]
29	10	2.8	6[illegible]	2[illegible]
30	6	1.3	3[illegible]	4[illegible]
31	8	2.0	4[illegible]	3[illegible]

blood five times per year regularly for many years. The latter group had a mean value of 6.4 ± 0.2 with a range of 4.8 to 8.2 *ng*/kg body wt. Study of bone marrow films also indicated that iron stores were depleted after therapy (Table IV). Histological study of liver biopsy specimens *after therapy was performed in two of the present* patients only (nos. 13 and 20) Patient 13 who had comparatively small iron stores before therapy (0.5 g of iron could be mobilized from stores), had no stainable iron in liver biopsy specimen after therapy *In* patient 20, who had large iron stores before therapy (mobilizable iron stores were 3.8 g), a few large hemosiderin granules could be demonstrated in histiocytic liver cells, but there was no iron pigment to be seen in the parenchymal liver cells. *Before* therapy these had been heavily loaded with hemosiderin.

Most patients tolerated the treatment well but many felt tired during the sideropenic state On the other hand, some patients maintained that they felt healthier during and after therapy than before.

Clinical remission and a markedly reduced urinary porphyrin excretion occurred in all. Skin fragility blisters and ulcers usually vanished 2 to 6 months after start of therapy The disappearance of skin fragility and ulcers roughly coincided with the time when uroporphyrin excretion decreased to a level of approximately 1 mg. Hyperpigmentation and hypertrichosis diminished less rapidly but were usually absent within one year from the start of therapy In one patient (no. .4) small blisters and pruritus persisted on the forearms for some months in spite of a urinary porphyrin excretion of only $^1/_2$ mg/day

A marked decrease in urinary porphyrin excretion (Fig. 3 Table VI) occurred in every patient, even in two alcoholics (nos. 15 and 16) who were seldom sober when seen in the Out patient Department. In 18 patients treated by frequent phlebotomy and who were not given iron medication during treatment, the urinary uroporphyrin excretion decreased to 1 mg/day or below this level within $^1/_2$ year (Fig. 3) Of these 18 patients alcohol consumption was judged to be unchanged in nine (nos. 13 to 20 and no 9), while it was judged to be more or less decreased during treatment in the other nine (nos. ?4 to 31 and no. 8). The mean period required until urinary porphyrin excretion decreased to 1 mg in the former was 4.5 ± 0.3 months, but in the latter only 3.4 ± 0.3 months.

As shown in Fig. 4 there was a significant relationship between the period required untill the urinary porphyrin excretion fell to 1 mg and until phlebotomy caused depletion of iron stores. The correlation coefficient 0.87 was highly significant ($p < 0.001$). There were however large variations. In some patients remission occurred some month after iron stores had been exhausted, and in other patients remission was obtained long before iron stores were depleted (Table V). In two patients remission did not occur until after 8 $^1/$ (no. 23) and 16 months (no. 22). In the former iron stores were not depleted until 8 months after start of therapy because phlebotomy could only be performed once or twice monthly In the latter iron was given orally in conjunction with phlebotomy for 6 $^1/_2$ months, and iron stores were not exhausted until 16 months after start of therapy In

Table VI. *Porphyrin excretion in phlebotomy-treated patients*

In five patients (nos. 13, 21 27 8 and 31) iron stores were replenished after phlebotomy. In all biochemical relapse occurred, and the figures in italics represent increased uroporphyrin excretion after iron replenishment. They were subsequently phlebotomized again and biochemical remission ensued in all. These patients will be described in detail in following report (17). In two patients (nos. 22 and 23) uroporphyrin excretion remained at high level for longer periods than in the others. In one of them (no. 22) oral iron was given in conjunction with phlebotomy for ½ year. In the other (no. 23) phlebotomy was performed less frequently (once or twice monthly). In patients not given iron, biochemical relapse occurred in one (no. 18)

Pat. no.	ALA (mg/24 h)	PBG	Urine: Uroporphyrin (µg/24 h) Before	½ y[a]	1 y	1½ y	2 y	3 y	4 y	CP (µg/24 h) Before	After[b]	Faeces: CP (µg/g dry wt) Before	After	PP (µg/g dry wt) Before	After
13	2.4	1.1	2 800	820	430	*910*	*4 720*	270		110	150	87[c]	5	85[c]	21
14	5.2	0.4	3 800	320	180	250	100	60	0 (11)[d]	510	50	128[c]	22[c]	129[c]	25[c]
15			5 900	800						600			9		7
16	0	0.8	6 800	270	330 (184)					260	80	25	20	18	29
17	0	0.9	3 900	350	250		180 (90)			140	40	7	8	27	15
18	3.1	0	6 900	1 000	240	200	330	7 400		340	100		2		8
19	1.1	0	4 700	690	700	600				310	100	10	9	14	10
20	2.2	0.8	4 100	540						380	30		19		11
9	0.5	0.6	4 500	900	300 (170)					200	130		26		37
21	1.4	1.5	2 400	590	260	*1 610*	520	490 (381)		250	140	8	13	15	13
22	0.6	0.6	11 000	5 400	2 100	530	280	50	30 (0)	530	20	134[c]	13	186[c]	101
23	2.5	0	4 700	4 100	870	350	200	120 (59)		380	100	352[c]	6	311	14
4	0	0.6	2 100	420	270	100 (53)				190	100	5	7	6	21
25	1.7	0.3	3 600	790	320			80 (89)		130	30	78[c]	5	83[c]	30
8	1.0	0.7	2 800	140	90		130	80	200 (22)	360	80		2		3
26	3.1	0	5 200	320	310	80	80	210 (124)		160	20	158[c]	8[c]	127[c]	25[c]
27	1.5	1.0	2 800	630	*1 000*	*7 100*	270 (225)			140	120	55	7	30	16
28	4.2	1.2	1 500	390	290	*5 600*	*830*	160	190 (15)	460	160	80[c]	13[c]	73[c]	7[c]
29	1.7	0.5	2 300	260	180			110 (81)		230	40	115[c]	9	92[c]	20
30	0.6	0	5 800	660	410					180	70	53	28	64	97
31	2.0	0	3 400	310	160	220	980	550		200	40		5		10
Upper normal limit					200 (25) µg/24 h					150 µg/24 h		10 µg/g		40 µg/g	

[a] Period after start of phlebotomy therapy
[b] After medium period after start of therapy of ½ year
[c] Analyses performed by Dr B. Haeger-Aronsen, Allmänna Sjukhuset, Malmö.
[d] Analysis performed according to Dresel et al (5)

Table VII. *Liver function tests before and after venesection therapy*

Pat. no.	SGOT (U.)		SGPT (U.)		Bilirubin (mg/100 ml)		BSPR (%)	
	Before	After	Before	After	Before	After	Before	After
Subjects with unchanged alcohol consumption								
13	44	24	65	35	0.5	0.6	6	3
14	60	39	55	25	1.4	0.7	19	11
15	58		50		0.4			
16	72	37	82	36	0.7	0.9	12	10
17	26	23	58	38	0.6	0.6	7	3
18	56	19	75	49	0.7	0.4	10	6
19	45	28	36	20	0.4	0.4	24	11
20	72	20	101	33	0.6	0.5	20	10
9	25	16	29	21	0.3	0.3	7	4
21	44	21	48	18	0.6	0.2	8	6
22	51	30	69	32	0.7	0.2	9	5
23	40	43	24	48	0.3	0.4	10	9
Subjects with decreased alcohol consumption during treatment								
24	23	15	46	25	0.3	0.3	2	11
25	65	21	80	23	0.5	0.3	14	3
8	110	88	117	107	1.1	0.7	34	11
26	37	29	61	14	0.5	0.4	16	7
27	41	25	50	25	0.5	0.4	14	5
28	72	24	87	20	1.1	0.4	3	1
29	44	40	25	20	1.0	0.4	10	6
30	69	20	120	30	0.6	0.8		1
31	85	45	80	39	1.4	1.2	25	10

this patient (Fig. 5) more blood had to be removed (22½ l) than in any other patient before remission could be achieved. One patient (no. 21) was given iron dextran intravenously during ten weeks intensive phlebotomy. The amount of iron administered after each phlebotomy was equal to the calculated iron content of the removed blood. The porphyrin excretion diminished temporarily during intensive phlebotomy but later increased. Iron administration was stopped and further phlebotomy caused iron depletion, and with this remission occurred.

The urinary coproporphyrin excretion which before treatment usually was only slightly increased (Table VI), became normal within ½ year. Also faecal porphyrin excretion decreased, and in many patients it became normal.

The liver function tests improved too (Table VII). Before treatment the SGOT and/or SGPT level was slightly to moderately increased in all but three patients. After treatment the transaminase levels were within normal limits in all but three patients. The BSPR values after treatment decreased in all but one.

The patients were followed for periods extending up to 4 years (Table VI, Fig. 6) after start of therapy. In five patients (nos. 13, 21, 27, 28 and 31) iron stores were repleted after remission had been induced, in all of whom biochemical relapses ensued. These patients will be described in detail in a following report (17). One additional

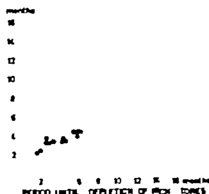


Fig. 4. Relation between the period (x) required until phlebotomy caused exhaustion of mobilizable iron stores and the period (y) after start of phlebotomy therapy during which the urinary uroporphyrin excretion remained at a level exceeding 1 mg/day. The correlation coefficient (0.87) was significant ($t = 7.6$, $p < 0.001$). The regression line represents the following equation: $y = 0.81 + 0.6x$.



Fig. 5. Effect of phlebotomy in patient 22, who received 300 mg of iron (as ferrous sulphate) orally per day for 6 / months in conjunction with phlebotomy. This patient had the longest treatment period. In all 22 / 1 of blood was removed and remission did not occur until 16 months after start of therapy. The term urine iron denotes the iron content in urine during 24 h after i.m. administration of 10 mg desferrioxamine per kg body weight. The hatched area represents the decreasing amount of storage iron available for hemoglobin production.

patient (no. 18) had a biochemical relapse (see below). In the rest of the patients the urinary porphyrin excretion remained low (Table VI) after 1 $^{1}/_{2}$ to 2 years ranging from 0.08 to 0.60 mg/day and after 3 to 4 years from 0 to 0.21 mg/day. The uroporphyrin values given above refer to urines in which ether soluble porphyrins were not extracted before the determination. In urines with small amounts of porphyrins the method of Dresel et al. (5) was also used (values within brackets in Table VI) for the determination of uroporphyrin. In urines from eight patients studied 2 to 3 / years after the last phlebotomy the uroporphyrin content determined according to Dresel et al. (5) ranged from 0 to 1.4 μg/24 h, and values within the normal range (0 to 25 μg/24 h) were encountered in four of them.

The results of the study of hepatic porphyrin fluorescence according to the technique described by Lundvall and Enerbäck (18) are shown in Table VIII and Fig. 7. In non-treated patients the grade of porphyrin fluorescence usually was 3+ but grades 2+ and 4+ occurred. In general the fluorescence had decreased after therapy but single patients had high grades of red fluorescence in spite of a low porphyrin excretion. Although repeated examinations for periods extending up

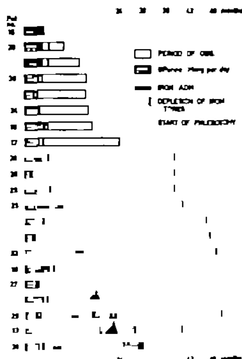


Fig. 6. Graph illustrating the length of the observation period after start of phlebotomy, the period required until phlebotomy caused depletion of iron stores and period with uroporphyrin excretion exceeding 1 mg/day. In five patients iron stores were repleted after phlebotomy and in all biochemical relapse ensued. These are illustrated at bottom of figure. Relapse also occurred in patient 18, who did not receive iron medication.

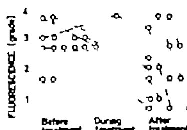


Fig 7 Hepatic fluorescence in fine-needle aspiration biopsy smears as related to treatment. Observations in the same patient connected by interrupted lines.

to 30 months indicated that the hepatic porphyrin fluorescence decreased with time (Fig. 7) It never vanished completely

A biochemical relapse occurred in one patient who had not been given iron supplements after therapy The course in this patient is described below

CASE REPORT

Patient 18, engineer born in 1917 (Fig. 8). No family history of diabetes mellitus or PCT He never consumed much alcohol. In recent years his alcohol consumption had been minimal, not exceeding 200 ml of hard liquor per month.

Table VIII *Hepatic porphyrin fluorescence in fine-needle aspiration smears before and after phlebotomy therapy*

Pat. no	Date	Relation to phlebotomy	Period after last phlebotomy (mo.)	Fluorescence (grade 1–4+)	Urinary uroporphyrin excretion (µg/24 h)
Nonphlebotomy-treated patients					
2	Aug 1968			3+	5 400
4	Nov 1968			3+	4 940
5	Sep 1968			2+	4 520
6	Sep 1968			2+	2 200
7	Sep 1968			3+	2 600
9	Feb 1968			3+	5 330
	Nov 1968			3+	5 190
Phlebotomy-treated patients					
13	Apr 1968	After	0	2+	530
14	Apr 1968	After	19	1+	100
15	Nov 1967	Before		4+	5 920
	Apr 1968	During		3+	1 300
16	Sep 1968	Before		3+	6 790
	Feb 1969	After	1	2+	410
17	Oct 1967	Before		3+	4 020
	Jun 1968	After	0	1	930
18	Mar 1968	After	7	1+	240
19	May 1968	Before		3+	9 190
20	Nov 1967	Before		3+	6 360
	Feb 1569	During		4	3 820
	Mar 1968	During		3+	2 200
	May 1968	After	0	2+	690
	Jun 1968	After	1	1	600
1	Feb 1968	After	6	2+	370
22	Oct 1967	After	5	4	200
	Mar 1968	After	10	3+	200
	Nov 1968	After	17	2	0
23	Jan 1968	After	9	4	410
	Oct 1968	After	18	3	120
24	Aug 1968	Before		3	3 710
	Feb 1969	After	2	1+	390
26	May 1968	After	11	1	80
27	Oct 1967	Before		4+	2 790
	Feb 1968	After	0	2	820
28	Dec 1967	After	3	2+	620
	Mar 1970	After	30	1+	190

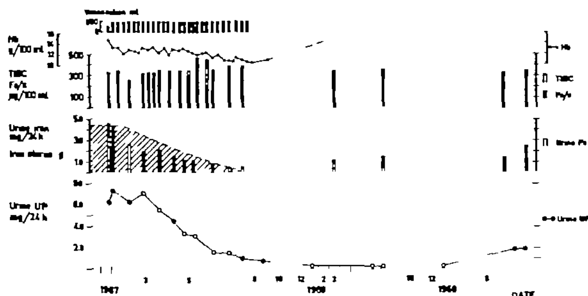


Fig. 8. Effect of phlebotomy in patient 18 who had the largest amount of mobilizable iron (4.4 g) of all treated patients. This patient had a biochemical relapse approximately two years after the initial phlebotomy series. Note the increasing level of the desferrioxamine-induced urinary iron excretion.

Diabetes mellitus was diagnosed in 1962 and was treated initially with diet. From Feb. 1965 he was given tolbutamide (1.0 g daily).

Excoriations, ulcers and blisters appeared on the backs of the hands in May 1965. The lesions followed minor trauma and healed slowly. Blistering occurred especially after exposure to sun, and the skin disease was less active in the winter although skin fragility persisted. He never noted red urine. He thought that the disease might represent hypersensitivity to tolbutamide but he did not stop this medication.

He was admitted in Feb. 1967. The face and neck had a violaceous hue and the uncovered skin areas were hyperpigmented. There was an increased hair growth on the face (especially on the temples). The conjunctivae were injected. There were numerous depigmented superficial pink scars on the backs of the hands. The liver was palpable at the costal margin on inspiration.

Determination of urinary uroporphyrin excretion (UUP) showed values of 6 330 to 7 450 µg/day. Urinary coproporphyrin (UCP) excretion was slightly increased (340 µg/day). Excretion of ALA was 3.1 mg/day but no PBG could be detected.

Liver biopsy showed marked periportal fibrosis, moderate steatosis (planimetric determination showed that 10% of the area consisted of fat vacuoles) and moderate periportal round cell infiltration. Laboratory study of liver function revealed a normal serum bilirubin (0.6 to 0.7 mg%) but increased BSPR (8 to 12% retention of 5 mg BSP/kg body wt after 45 min) and raised transaminases (SGOT 56 and 56 U, SGPT 70 and 80 U). Alkaline phosphatase in serum was slightly increased (11 to 12 Bock units). Fasting blood glucose level ranged from 165 to 215 mg/100 ml. ESR was 16 mm/h.

Haemoglobin concentration (14.3 to 14.0 g%) was normal. Iron studies showed a high serum iron level (300 µg%), a high TIBC saturation (91%), moderate amounts of reticular bone marrow iron (grade 2 in sections, grade 3 in smears) and a high sideroblast count (80%). In the liver biopsy specimen there were very large amounts of iron pigment in the parenchymal cells (grade 4+) and there was also hemosiderin in most Kupffer cells (grade 3+). Determination of liver non-heme iron showed a value of 600 mg/100 g dry weight and 1 450 mg/100 g liver protein. The desferrioxamine-induced urinary iron excretion was 4.33 mg (or 62 µg/kg body wt). At phlebotomy 4.4 g of iron was mobilized from iron stores.

Phlebotomy therapy was started in Feb. 1967 (Fig. 8). Alcohol consumption and tolbutamide medication were unchanged during therapy. Fourteen l of blood were removed, and iron stores available for hemoglobin synthesis were judged to be exhausted in Sept. 1967 (Fig. 8). He tolerated the phlebotomy therapy well and maintained that he felt stronger and healthier during and after therapy than before.

Uroporphyrin excretion (Fig. 8) remained at a high level during the first 1 / months' treatment. Then it gradually decreased, and in Aug. 1967 it fell to less than 1 mg/day. From Jan. 1968 to Jan. 1969 it varied between 200 and 390 µg/day.

Skin fragility persisted and occasional blisters appeared in the summer 1967 but then gradually decreased and vanished completely in the autumn of 1967. Hyperpigmentation and hypertrichosis were absent one year after therapy.

Laboratory study of liver function after therapy (in Aug. 1967) indicated improvement (bilirubin 0.4 mg%

with parenchymal liver cells loaded with iron pigment. In the liver biopsy specimen obtained after phlebotomy there were a few large hemosiderin aggregates in Kupffer cells, but no iron could be demonstrated in the parenchymal liver cells.

Not only was the size of mobilizable iron stores normal in some of the present patients, but several had a normal liver iron concentration as determined chemically (23) The phlebotomy therapy was effective also in patients without iron overload. Nevertheless, the results of the present study favour the view that the effect of phlebotomy therapy is related to the reduction of iron stores. A significant relationship between the period required to achieve iron depletion and to achieve biochemical remission (Fig. 4) was found. The correlation coefficient for this regression was high, but there were large individual variations. These may be due partly to variations in exposure to porphyrogenic substances, *e.g.* alcohol and drug ingestion. Another cause of discordance of the time relation between reduction of storage iron and remission might be variations in the porphyrin content of the liver As shown by Schmid et al. (31) the liver in porphyria cutanea tarda contains large amounts of preformed porphyrins (mainly uroporphyrin) but with large variations between individuals. In patients with active disease the livers contained from 3 to 78 mg of uroporphyrin per 100 g. It seems reasonable to assume that remission may occur later in patients who have a very high concentration of porphyrins in the liver In such cases the urinary porphyrin excretion may perhaps remain on a high level for a long period in spite of an improved porphyrin synthesis.

The results of iron administration in conjunction with phlebotomy also indicate that phlebotomy is effective by reduction of iron stores. In the patient (no. 2.) who was given oral iron substitution for 6 $^1/_2$ months the treatment period was the longest, and the amount of blood that had to be removed in order to achieve remission was the largest of all patients treated. In the patient given parenteral iron in conjunction with phlebotomy the results are less conclusive because iron administration was stopped after _ months, since the urinary porphyrin excretion increased.

If phlebotomy is effective by reducing iron stores, this implies that relapse may occur if iron stores are repleted. In five of the present patients iron stores were repleted by oral or parenteral iron administration. As will be shown in a following report (17) biochemical relapse occurred in all (see also Fig. 6 in the present paper). Fourteen of the present patients not given iron after phlebotomy-induced remission were observed for periods of 1 to 4 years (Fig. 6). In only one of them was there a relapse. In this patient (no. 18) the relapse occurred after a remission period of two years. The desferrioxamine test indicated that he had reaccumulated iron stores of considerable size.

In some reports the effect of phlebotomy therapy was equivocal. This may have been due to the iron stores not having been sufficiently reduced. Káldor et al. (14), for instance, who failed to achieve chemical remission in four out of six patients, removed total amounts of only 0 6 to 3 4 l of blood.

ACKNOWLEDGEMENT

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THE EFFECT OF REPLENISHMENT OF IRON STORES AFTER PHLEBOTOMY THERAPY IN PORPHYRIA CUTANEA TARDA

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Abstract. In previous study phlebotomy therapy was consistently effective in porphyria cutanea tarda, causing clinical and biochemical remission in all. In 13 patients observed for periods of 1 to 3 / years after treatment and who were not given iron, relapse occurred in only one patient. The latter had reaccumulated iron stores of considerable size spontaneously In the present study the effect of replenishment of iron stores after phlebotomy-induced remission was investigated. Five patients (4 men and 1 woman) were included, in four of whom the initial iron stores (before phlebotomy) had been of normal size. In one of them moderate iron overload had been present. Iron stores were replenished by oral administration of iron sulphate in four and by intravenous administration of iron dextrin in one. After rep.enishment of iron stores biochemical relapse occurred in each patient. The skin disease remained in remission, except in the woman who noted an increased hair growth on the face. They ere again subjected to repeated phlebotomy Biochemical remission occurred in all and the hyper trichosis in the woman vanished. It is concluded that phlebotomy probably exerts its effect by reduction of iron stores, and that even normal amounts of storage iron may provoke deterioration of porphyrin metabolism in subjects with latent disease.

Since Ippen introduced phlebotomy therapy of porphyria cutanea tarda (PCT) in 1960 (6) several reports have appeared indicating that this therapy is effective (for review see (10)). In most of these, however control series were lacking and abstinence of alcohol was part of the treat ment. In a study of the effect of phlebotomy treat ment of PCT *by the present author (10) the al*cohol consumption was unchanged in part of the treated series and a control group was included. It was confirmed that phlebotomy therapy does cause remission in PCT Clinical and biochemical remission occurred consistently in 21 treated patients, even in those with persistent abuse of alcohol. In a control series of 1.. patients, biochemical improvement comparable to that in the phlebotomy-treated series occurred in only two. It was suggested (10) that phlebotomy exerts its effect by reduction of iron stores, since there was a significant relationship between the period elapsing until phlebotomy caused remission and until phlebotomy caused depletion of iron stores.

In the present investigation the significance of the size of iron stores for the clinical and biochemical manifestations of PCT was studied by replenishment of iron stores after phlebotomy induced remission.

MATERIAL AND METHODS

In five patients with porphyria cutanea tarda treated with phlebotomy until signs of depletion of iron stores appeared and biochemical and clinical remission occurred, iron stores were replenished by oral iron medication (4 patients) or by parenteral iron administration (1 patient). Iron stores ere quantitated at intervals by the desferriox amine test. Iron stores available for hemoglobin synthesis (mobilizable iron stores) were determined at phlebotomy therapy

The effect of the building up of new iron stores on the porphyrin metabolism was studied by determination of the porphyrin excretion in urine and faeces. Also liver function was studied before and after replenishment of iron stores. The methods used in this study were the same as employed in previous investigation (10). Unless otherwise stated, values given for urinary uroporphyrin excretion represent determinations performed according to the technique of Askevold (1).

The following abbreviations will be used: UUP = urinary uroporphyrin (N V = normal value < 200 μg/24 h), UCP = urinary coproporphyrin excretion (N.V < 150 μg/24 h), PBG = urinary porphobilinogen (N.V < 2 mg/24 h), ALA = urinary delta-aminolevulinic acid (N V < 5 mg/24 h), FCP = faecal coproporphyrin (N.V < 10 μg/g

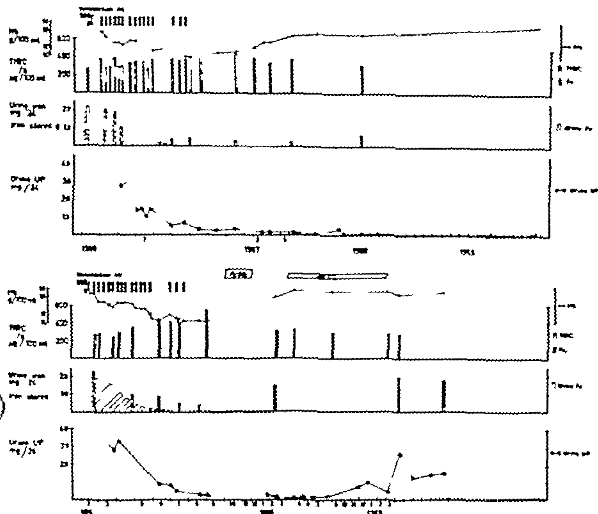


Fig 1 The response to phlebotomy in the uniovular twins case 2 (top) and case 1 (bottom), and the effect of oral replenishment of iron stores in the latter In case the urinary porphyrin secretion remained low during an observation period of mor than 3 years. In case 1 urinary porphyrin excretion showed significant increase hhin 1 / year after phlebotomy The term urine iron denotes the iron content in urine during 4 h after i.m. administration of 10 mg f desferrioxamine per kg body eight. *The hatched areas epresent iron stores available for hemoglobin synthesis at phlebotomy*

dry t), FPP = faecal protoporphyrin excretion (N.V <40 µg g dry t) SB = serum bilirubin (N.V <1.1 mg 100 ml), AP = alkaline phosphatase (N.V <8 units), Th = thymol turbidity (N.V extinction <0.10), SGOT (N.V <40 units), SGPT (N.V <40 units), BSPR = bromsulphalein retention determined 45 min after the administration of 5 mg of the dye kg body t (N.V <5%), Fe/urine = iron content in urine collected for 4 h after i.m. injection of 10 mg f desferrioxamine kg body wt (N.V for men <1.22 mg or 14.3 µg kg body wt).

CASE REPORTS

Case 1

Patient 31 (ref. (10)) (Fig. 1 bottom) Male commercial traveller born in 1917 Twin, probably identical, of the following patient (case 2). They look and behave very much alike. These twins ere studied by Waldenström and Haeger Aronsen in 1959 and ere found to have identical blood groups: A., MN P K+ Rh− Hp — In 1959 case 1 had the following porphyrin excretion: UUP 77 µg 100 ml, UCP 10 µg 100 ml, FCP 1 µg g dry t, FPP 20 µg g dry wt (cf 1).

When examined in the Outpatient Service at Medical Department I, Sahlgren's Hospital, in Feb. 1967 the skin disease had low grade of activity: the patient had noted skin fragility for some years and single blisters on some occasions. He had been heavy consumer of alcohol for at least 20 years. During the years immediately before the study he had a rather regular consumption of 2 to 3 l of hard liquor per eek. H had not noted any connection bet een alcohol consumption and skin symptoms but maintained that his urine could be darker on the day after heavy alcohol consumption.

Table I. *Liver function tests and DF-induced urinary iron excretion in case 1. Compare with Fig. 1*

	Upper limit of normal	Date (month, year) II 1967	VI, 1967	IX, 1967	IV 1969	IX, 1969	I 1970
SB (mg/100 ml)	1 1	1 4	1.2	0.7	1.3	1.0	0.6
Th (extinction)	0.10	0 01	0 01	0.01	0.01	0 01	0.01
AP (U)	8	8	7	7	5	4	6
SGOT (U)	40	85	45	36	60	38	30
SGPT (U)	40	80	39	27	59	60	40
BSPR ( )	5	23	10				
Fe/urine (mg)	1.22	2.28	0 83	0 42	1.98	1 76	
Fe/urine (µg/kg body wt)	14.3	28.5	10 4	5.3	24 8	22.0	
UUP (µg/24 h)	200	2 900	830	310	2 600	1 510	590

At examination there are no recent ulcers or blisters but few superficial scars on the dorsae of the hands. There was slight hyperpigmentation on skin areas exposed to light but no hypertrichosis. The conjunctivae were injected. The liver was palpable 1–2 fingers below the costal margin on inspiration but the consistency was not increased.

Porphyrin studies showed greatly increased UUP (3 420 µg/24 h), but UCP was only slightly raised (200 µg/24 h). Excretion of ALA (2 0 mg/24 h) and PBG (0) as normal.

Intravenous glucose tolerance (k_g = 1.07) was low normal. Laboratory study of liver function showed slightly increased serum bilirubin, moderately increased serum transaminase levels and BSPR of 25% (Table I).

Iron studies showed normal serum iron concentration (156 µg%) but an increased desferrioxamine-induced iron excretion (Table I, Fig. 1).

Phlebotomy therapy was started in Feb. 1967 Alcohol consumption during treatment (about 6 1 of hard liquor per month) was somewhat decreased as compared with that before therapy and then remained essentially unchanged during the observation period. As shown in Fig. 1 sixteen venesections of about 0.5 1 each were performed. Four and half months after start of treatment the iron stores available for hemoglobin synthesis (2 g of iron) were exhausted, and there were persistent anemia, low serum iron, high total iron binding capacity and low DF-induced urinary iron excretion (Fig. 1). Urinary uroporphyrin excretion decreased to level of 1 mg within 3 / months after start of therapy The porphyrin excretion then decreased further (Fig. 1), and in Aug. 1967 the following porphyrin values were obtained: UUP 280 µg, UCP 38 µg/24 h, FCP 5 FPP 10 µg g dry t. The porphyrin excretion then remained low until the autumn of 1968. Laboratory study f liver function after therapy indicated improvement (Table I).

The patient tolerated the phlebotomies without untoward effects, but after therapy he had persistent tiredness and complained of palpitations on exertion. He as given 300 mg of iron (as ferrous sulphate) daily during Oct. and Nov 1968. The tiredness rapidly disappeared. Later iron administration was resumed, he was given 150 mg of iron (as ferrous sulphate) per day orally from April 1968 to Feb. 1969

As shown in Fig. 1 the urinary porphyrin excretion remained low until the autumn of 1968 but then increased. When studied in April 1969 the DF-induced iron excretion was of the same order as before phlebotomy (Table I). Serum iron was 158 µg/100 ml. In bone marrow smears normal amounts of reticular iron are found (grade 2+), but the sideroblast count was high (79%). The UUP as 2 600 µg/24 h. Laboratory study of liver function showed increased transaminase levels (Table I). In spite of the biochemical relapse no skin symptoms appeared.

A second course of phlebotomy as started in Sept. 1969 and was concluded in Jan. 1970 T elv phlebotomies of 0.5 1 ere performed, after which he had hemoglobin value of 13 4 g/100 ml (initial Hb level was 17 1 g 100 ml), low serum iron (35 µg 100 ml) and high transferrin level (465 µg/100 ml) The amount of iron mobilized from iron stores was approximately g, but iron stores vailable for hemoglobin synthesis might have been greater because phlebotomy as not carried far enough to cause reliable signs f iron deficiency Porphyrin excretion gain decreased, and in Jan. 1970 porphyrin analyses gave the following results: UUP 550 µg and UCP 135 µg/24 h.

Case 2

Patient 14 (ref. (10)) (Fig 1 top) Male commercial traveller born in 1917 T in of case 1 He has had large and regular alcohol consumption since he as 25 years old The average alcohol ingestion in recent years as estimated to be at least 4 1 of hard liquor per month.

This patient ith porphyria cutanea tarda was treated by phlebotomy until remission and depletion of iron stores occurred. He as not given iron after therapy The course is reported in some detail because he is probably the identical twin of the foregoing patient and should represent an ideal control of the latter

He had attacks of abdominal pain which disappeared after appendectomy in 1934.

Skin fragility ulcers and blisters on skin areas exposed to the sun appeared in 1952. The lesions usually healed in the winter. In Oct. 1959 he was given chloroquine for the skin disease and had typical chloroquine reaction (19). After one or two days' oral administration

Table II. Liver function tests and DF-induced urinary iron excretion in case 2. Compare with Fig. 1

	Upper limit of normal	Date (month, year)			
		IV, 1966	XII, 1966	I, 1968	IX, 1969
SB (mg/100 ml)	1.1	1.4	0.5–0.8	0.7	0.8
Tb (extinction)	0.10	0.06	0.01	0.02	0.01
AP (U)	8	7	7	5	5
SGOT (U)	40	60	39	20	25
SGPT (U)	40	55	25	25	30
BSPR (%)	5	19	10–12		
Fe urine (mg)	1.22	2.1	0.34	0.70	0.63
Fe urine (µg/kg body wt)	14.3	28.3	4.4	9.0	8.1
UUP (µg/24 h)	200	3 800	240	110	110

of the drug, he fell acutely ill and was admitted to hospital because of pyrexia, malaise, headache, abdominal and muscular pain. The urine was dark. SB was 4.3 mg% and SGPT 128 units. Porphyrinuria was demonstrated. He recovered from the acute illness in one week. He was studied by Waldenström and Haeger-Aronsen in Dec. 1959. The following porphyrin values were obtained: UUP 167 µg/100 ml, UCP 31 µg/100 ml, FCP 68 µg/g dry wt, FPP 27 µg/g dry wt (21).

During the summers 1960–1964 the blistering was prominent but not thereafter. However, the skin fragility persisted and excoriations on the hands followed trivial trauma.

When studied in April 1966 there was a general hyperpigmentation and hypertrichosis. The hypertrichosis was prominent on the face, and the hair margins at the temples were diffuse and extended down to the eyebrows. There were several superficial pink scars on the backs of the hands. The conjunctivae were injected. The liver was palpable 3 finger breadths below the right costal margin on inspiration. The consistency of the liver was normal. Liver biopsy (in 1960) had shown slight fibrosis, steatosis and periportal round cell infiltration. Porphyrin analysis showed a grossly increased urinary uroporphyrin excretion (UUP 3 180 to 4 400 µg/24 h), while UCP was moderately increased (510 µg/24 h). Excretion of ALA (5.. mg/24 h) was at the upper limit of normal, and excretion of PBG was normal (0.4 mg/24 h). Faecal porphyrin excretion was moderately increased (FCP 128, FPP 179 µg/g dry wt).

Laboratory study of liver function indicated active liver disease with moderately raised transaminase levels (Table II). Intravenous glucose tolerance was decreased (k_g = 0.83). Hemoglobin concentration was normal (15.9 g%). Iron studies showed a high serum iron level (243 µg%). Initially DF-induced urinary iron excretion was increased (Table II). In bone marrow smears there were plentiful amounts of reticular hemosiderin (grade 3+) and the sideroblast count was 81%. In liver biopsy specimen from 1960 stainable iron of grade 3+ was present both in parenchymal and histiocytic cells.

Phlebotomy therapy was started in April 1966. The alcohol consumption was essentially unchanged during the study (about 1.1 of hard liquor per week).

Fifteen phlebotomies were performed. As shown in Fig. 1 (top) these produced signs of depleted iron stores. The hemoglobin production was curtailed and there was persistently low serum iron level. Also the DF-induced urinary iron excretion was low.

The iron stores available for hemoglobin production amounted to ..0 g and were depleted 4 months after the start of therapy (Fig. 1).

The urinary porphyrin excretion showed a marked decrease one month after start of therapy diminishing to 1 mg 3 1/ months after start of phlebotomy and then further decreasing. In Dec. 1966 porphyrin analysis gave the following results: UUP .40 µg, UCP 40 µg/24 h, FCP 22 µg and FPP .5 µg/g dry wt. The porphyrin excretion then remained at low level (Fig. 1 top). Hepatic fluorescence was studied in fine-needle aspiration smears in April 1968 (19 months after the last phlebotomy) and showed weak red fluorescence in some hepatocyte nuclei (grade 1+). The UUP at that time was low (100 µg/24 h). When last studied (Feb. 1970) the following porphyrin values were obtained: UUP 0, UCP 7? µg/24 h and FCP 5, FPP 1 µg/g dry wt.

Skin fragility vanished in the summer of 1966. Also the hypertrichosis as well as the hyperpigmentation gradually disappeared.

According to laboratory studies, liver function was improved after therapy (Table II). Intravenous glucose tolerance was again determined in Dec. 1966 and also showed improvement (k_g = 1.25).

The DF-induced urinary iron excretion remained at low level eight months after the end of therapy. It then increased to normal level and when last studied, in Sept. 1969, it was 0.63 mg (8.1 µg/kg).

Case 3

Patient 28 (ref. (10)) (Fig. ., Table III). Woman born in 1977. An alcoholic brother has manifest PCT and three asymptomatic siblings were found to have fluorescence of porphyrin type in fine-needle aspiration biopsy smears from the liver. This patient never abused alcohol, but rather regularly consumed 100–150 ml of hard liquor at weekends for many years. Since Sept. 1963 she has abstained from alcoholic drink totally.

She noted hypertrichosis of the face in the spring of

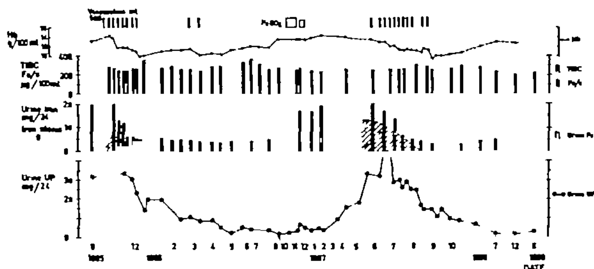


Fig. 2. The effect of phlebotomy and of oral replenishment of iron stores in case 3.

1963 and skin fragility with slowly healing ulcers on the dorsae of the hands in the summer of the same year. Blisters occurred on some occasions in summertime but without evident association with exposure to the sun. She noted dark urine on occasions since 1964.

She was studied in Sept. 1965. She had ulcers and typical pink scars on the forearms, backs of hands and on the ankles. There was a marked fine downy facial hypertrichosis. The liver was not palpable.

Excretion of ALA (4.2 mg/24 h) and PBG (1.2 mg/24 h) was normal, UUP was grossly increased (3 220–3 510 µg/24 h), while UCP was slightly increased (460 µg/24 h). Also faecal porphyrin excretion was raised (FCP 80, FPP 73 µg/g dry wt).

Liver function studies showed high serum transaminase levels but the BSPR was normal (Table III). Intravenous glucose tolerance was decreased ($k_e = 0.64$).

Iron studies showed a high serum iron level (191–222 µg%). The DF-induced urinary iron excretion was considerably increased (Table III). Phlebotomy therapy was started in Nov. 1965. After seven weekly phlebotomies there were curtailed hemoglobin production, low serum iron and low DF-induced iron excretion, indicating that iron stores were depleted. In bone marrow smears, however, single hemosiderin granules were present and the sideroblast count was 10%. Hence she was phlebotomized twice again in March 1966 (Fig. 2) but no more iron could be mobilized from iron stores. Iron stores available for hemoglobin synthesis were used up by the end of Jan. 1966 and amounted to 0.7 g. Urinary uroporphyrin excretion decreased gradually during phlebotomy and reached a level of 1 mg at the end of Feb. Active skin symptoms vanished at about the same time. Hypertrichosis disappeared gradually in the spring and summer of 1966. Liver function tests improved and became normal (Table III). In Oct. 1966 oral iron administration was started. She was given 400 mg of iron (as ferrous sulphate) daily for 30 days and then 300 mg daily for 15 days. As shown in Fig. 2, the DF-induced urinary iron excretion after the iron medication reached the same level as before phlebotomy. The first months after the iron administration the UUP remained essen-

Table III. *Liver function tests and desferrioxamine-induced iron excretion in case 3. Compare with Fig. 2*

	Upper limit of normal	Date (month, year) IX, 1965	I, 1966	XII, 1966	V, 1967	VIII, 1967	IX, 1969	III, 1970
SB (mg/100 ml)	1 1	0 6–1 6	0 2	0 4	0.5	0.3	0.4	0 5
Tk (extinction)	0.10	0 02–0 04	0 01	0 03	0 05	0 01	0 01	0 02
AP (U.)	8	6–7	7	6	7	6	6	6
SGOT (U.)	40	68–80	56	24	25	20	19	12
SGPT (U.)	40	78–105	40	25		45	20	25
BSPR ( )	5	3–3	1	1				
Fe/urine (mg)	1.22	1.89	0.53	1.69	1.38	0 50	0.93	
Fe/urine (µg/kg body wt)	14 3	28.6	8.0	25.6	30 0	7 6	14.1	
UUP (µg/24 h)	200	3 220	2 000	690	1 870	2 500	330	150

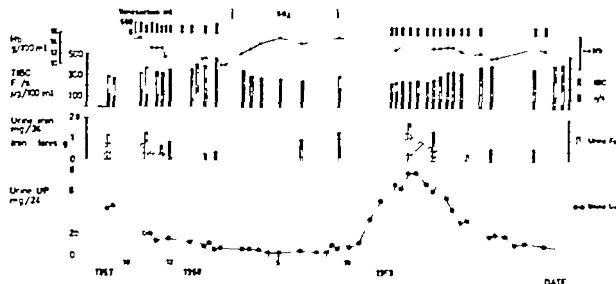


Fig 3 Response to phlebotomy therapy and oral iron replenishment in case 4

tially unchanged, but showed a definite increase in March 1967 and reached high levels in the following summer (Fig. 3). Hypertrichosis again appeared but no other skin symptoms.

A new series of phlebotomies was started in June 1967. As seen in Fig. 3, eleven venesections of about 0.5 l each were performed. This time 1.3 g of iron could be mobilized from iron stores, which became depleted about 3 months after start of therapy. Urinary porphyrin excretion began to decrease after four phlebotomies and then *diminished to 1 mg day approximately 4 months* after the start of therapy. It then further decreased (Fig. 3). In May 1969 porphyrin analyses gave the following results. UUP 330 µg/24 h, FCP 7 µg and FPP 29 µg/g dry wt. UUP then remained at a low level, and when analysed according to the method of Dresel et al. (3) in Dec. 1970 a normal value (15 µg/24 h) was obtained. However fluorescence microscopy of smears from fine-needle aspiration biopsy in March 1970 still showed a weak fluorescence of porphyrin type in some of the hepatocyte nuclei (grade 1+).

The hypertrichosis vanished in the winter of 1967 1968 and since then she has had no skin symptoms.

The DF-induced urinary iron excretion was last determined in Sept. 1969 (one year after phlebotomy) and indicated that she had accumulated new iron stores, but not of the magnitude before therapy (Table III).

Laboratory study of liver function (Table III) showed normal values after the last phlebotomy treatment.

Case 4

Patient 27 (ref. (10)) (Fig. 3 Table IV). Male born in 189_. No family history of PCT. He had been a moderate consumer of alcohol since his youth. In recent years he had consumed approximately 1 / 1 of distilled spirits per month.

In 1918 he was treated with salvarsan injection because of luetic infection. Myocardial infarction in 1959. Effort angina before but not after the myocardial infarction.

Skin fragility and traumatic ulcers and blistering appeared on the back of the hand in 1966. Single blisters

Table IV *Liver function tests and desferrioxamine-induced urinary iron excretion in case 4. Compare with Fig 3*

	Upper limit of normal	Date (month, year) IX, 1967	II 1968	IX 1968	I 1969	XI 1969	II 1970
SB (mg/100)	1.1	0.4–0.5	0.4	0.7	0.8	0.7	0.5
Th (extinction)	0.10	0.11–0.12	0.4	0.3	0.21	0.17	0.22
AP (U.)	8	11 12	14	17	13	19	23
SGOT (U.)	40	5–45	25	31	35	[illegible]	18
SGPT (U.)	40	50–53	[illegible]5	40	53	15	[illegible]
BSPR ( )	5	11 17	5		9		
Fe urine (mg)	1.22	1.16	0.47	1.43		0.61	0.62
Fe urine (µg/kg body wt)	14.3	13.6	5.5	16.8		7.6	7.3
UUP (µg/24 h)	200	4 580	710	1 003	5 823	745	770

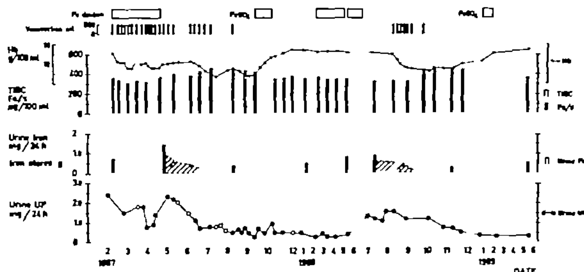


Fig 4 Response to phlebotomy therapy and oral iron replenishment in case 5

also appeared on the nose. Although the patient did not note any connection with exposure to the sun, the disease was very active in the summer of 1967 When studied in Sept. 1967 there were numerous old and recent scars, erosions and ulcers on the hands but no hypertrichosis or hyperpigmentation.

The liver was palpable on inspiration 3 fingerbreadths below the right costal margin. The consistency of the liver was normal. Liver biopsy showed moderate steatosis (planimetric determination showed that fat droplets constituted 7.8% of the area of the biopsy section) and slight periportal fibrosis.

Porphyrin analyses showed normal excretion of ALA (1.5 mg/24 h) and PBG (1.0 mg/24 h). UUP was grossly increased (4 580 µg/24 h) but UCP was normal (140 µg/24 h). FCP was increased (55 µg/g dry wt) but FPP was normal (30 µg/g dry wt). Fluorescence microscopy of aspiration liver biopsy smears showed strong red fluorescence both in nuclei and cytoplasm in virtually all hepatocytes (grade 4+).

Laboratory tests indicated impaired liver function (Table IV), but intravenous glucose tolerance was normal (k_g = 1.31) The hemoglobin concentration was normal (14 6 g/100 ml). Iron studies showed iron stores of normal size. Serum iron (129 µg/100 ml) and the TIBC saturation (42%) were normal, as was the DF-induced urinary iron excretion (Table IV). Liver non-heme iron concentration (98 mg/100 g dry wt, or 218 mg/100 g protein) was close to the average (104 mg/100 g dry wt or 186 mg/100 g protein) of control males (13). Histochemically demonstrable liver iron was of grade + in parenchymal cells and of grade + in Kupffer cells. In bone marrow smears reticular hemosiderin was graded 2+ and the sideroblast count showed value of 61%.

Phlebotomy therapy as started at the end of Oct. 1967 He stopped consuming distilled spirits in Aug. 1967 and has since then not consumed hard liquor but 1 2 glasses of beer daily

Eleven phlebotomies of 0 4 to 0 5 l ere performed (Fig 3). He tolerated the treatment well. Anemia, sideropenia and low DF-induced urinary iron excretion developed (Fig 3). At the end of Feb 1968 no reticular iron and no sideroblasts could be demonstrated in bone marrow smears. Iron stores available for hemoglobin production were judged to be depleted by the end of Jan. 1968 and ere calculated to hav been 0.8 g

Urinary porphyrin xcretion decreased during the tw months before therapy possibly due to decreased alcohol consumption. The UUP further decreased and fell to level of 1 mg at about the same time as the iron stores became depleted. In Feb 1968 the hepatic porphyrin fluorescence had decreased to grade +

Skin fragility gradually decreased and he has had no skin symptoms since Jan. 1968.

Liver function tests performed in Feb. 1968 showed normal transaminase levels and improved BSPR (Table IV).

He was given 90 mg of iron four times daily as ferrous sulphate from the middle of March to the middle of Sept. 1968 The DF-test (Fig 5) gradually increased, and in Sept. 1969 the DF-induced iron excretion showed somewhat higher value than that before the phlebotomy therapy (Table IV).

The urinary porphyrin excretion increased slightly in Aug. 1969 (after 5 months iron medication) and rose to high levels in the following months (up to 8 150 µg/24 h). Hepatic fluorescence studied in Jan. 1969 showed porphyrin fluorescence of grade 3+ The serum transaminases and the BSPR showed slight increases (Table IV).

A new series of phlebotomies was started in Jan. 1969 Fifteen phlebotomies of 0.4 l were performed. Iron stores available for hemoglobin synthesis (1.5 g) were depleted by the end of May 1969 UUP remained at high level during the first two months of phlebotomy but then decreased and reached level of 1 mg in July 1969

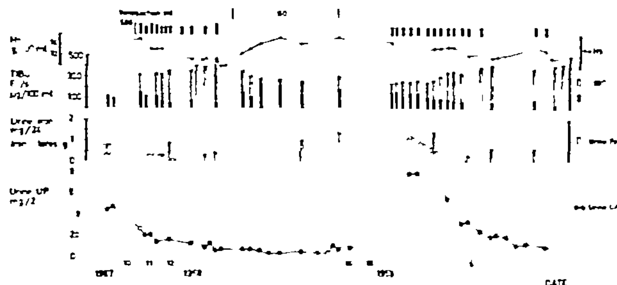


Fig 3 Response to phlebotomy therapy and oral iron replenishment in case 4

tially unchanged, but showed definite increase in March 1967 and reached high levels in the following summer (Fig. 3). Hypertrichosis again appeared but no other skin symptoms.

A new series of phlebotomies was started in June 1967 As seen in Fig. 3, eleven venesections of about 0.5 l each were performed. This time 1.3 g of iron could be mobilized from iron stores, which became depleted about 3 months after start of therapy. Urinary porphyrin excretion began to decrease after four phlebotomies and then diminished to 1 mg day approximately 4 months after the start of therapy. It then further decreased (Fig. 3). In May 1969 porphyrin analyses gave the following results: UUP 330 µg 24 h, FCP 7 µg and FPP 29 µg g dry wt. UUP then remained at a low level, and when analysed according to the method of Doss et al. (3) in Dec. 1970 a normal value (15 µg 24 h) was obtained. However fluorescence microscopy of smears from fine-needle aspiration biopsy in March 1970 still showed weak fluorescence of porphyrin type in some of the hepatocyte nuclei (grade 1+)

The hypertrichosis vanished in the winter of 1967 1968 and since then she has had no skin symptoms.

The DF-induced urinary iron excretion was last determined in *Sept. 1969 (one year after phlebotomy)* and indicated that she had accumulated new iron stores, but not of the magnitude before therapy (Table III)

Laboratory study of liver function (Table III) showed normal values after the last phlebotomy treatment.

Case 4

Patient 77 (ref. (1)) (Fig. 3 Table IV) Male born in 19[illegible]. No family history of PCT. He had been a moderate consumer of alcohol since his youth. In recent years he had consumed approximately 1 l of distilled spirits per month.

In 1918 he was treated with salvarsan injection because of luetic infection. Myocardial infarction in 1959. Effort angina before but not after the myocardial infarction.

Skin fragility and traumatic ulcers and blistering appeared on the backs of the hands in 1966. Single blisters

Table IV *Liver function tests and desferrioxamine-induced urinary iron excretion in case 4 Compare with Fig 3*

	Upper limit of normal	Date (month year) IX, 1967	II 1968	IX 1968	I 1969	XI 1969	II 1970
SB (mg 100)	1 1	0 4–0 5	0 4	0	0 8	0	0.5
Th (extinction)	0 10	0.11–0 12	0 [illegible]	0.3	0 [illegible]	0 1	0 22
AP (U.)	8	11 [illegible]	14	1	11	17	[illegible]
SGOT (U.)	40	[illegible]5–45	25	[illegible]	[illegible]	22	13
SGPT (U.)	40	50–50	[illegible]5	40	53	15	[illegible]
BSPR ( )	5	11 1	5		9		
Fe urine (mg)	1 22	1 16	0 47	1 43		0 61	0 [illegible]
Fe urine (µg kg body wt)	14 3	13 6	5.5	16 8		7 6	[illegible]
UUP (µg 24 h)	[illegible]	4 [illegible]	710	1 0[illegible]	5 [illegible]	745	[illegible]

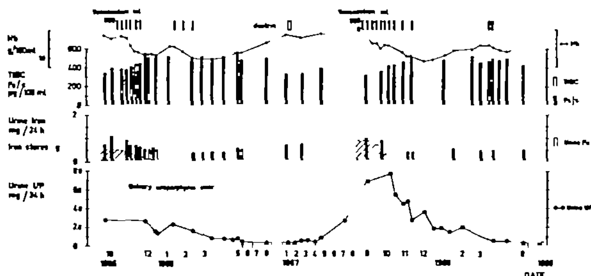


Fig. 5. Response to phlebotomy therapy and replenishment of iron stores with iron dextrin intravenously in case 6.

openia, low DF-induced iron excretion and absence of sideroblasts and reticular iron in bone marrow smears. Iron stores available for hemoglobin synthesis were used up by the end of October and amounted to 0.6 g. The urinary porphyrin excretion gradually decreased, and in June 1969 porphyrin analysis gave the following results: *UUP 370 µg/24 h, UCP 140 µg/24 h, FCP 13* and *FPP* 13 µg/g dry wt. The UUP has thereafter remained essentially unchanged. In Sept. 1969 the faecal porphyrin excretion was normal (FCP 9 FPP 34 µg/g dry wt). The transaminase level after the second series of phlebotomies again decreased and since then has remained normal. In Jan.–Feb. 1969 he was given 150 mg of iron (as ferrous sulphate) daily for 30 days because of tiredness and persistent anemia. The anemia was rapidly cured. He has thereafter apart from neurasthenic periods, enjoyed good health. He has had no skin symptoms. The DF test last performed in June 1969 showed low value (0.54 mg or 6.8 µg/kg). The UUP last determined in Feb. 1970 was 490 µg/24 h.

Case 6

Patient 13 (ref. (10)) (Fig. 5, Table VI). Male chauffeur born in 1912. There was no family history of PCT but the mother had diabetes mellitus. H had for many years regularly consumed about 4 l of distilled spirits per month. The alcohol consumption was judged to be essentially unchanged during the study

Ulcers and blisters on the backs of the hands and fingers and also on the nose and the ears appeared rather acutely in May 1965 after some days intensive exposure to the sun. Blistering was not prominent later, but skin fragility persisted and superficial erosions followed trivial trauma. On examination in Oct. 1965 there were several typical pink scars on the dorsum of hands and fingers. There were also fresh crusted ulcers. The hair growth was normal and there was no obvious hyperpigmentation. The liver was palpable 1– fingerbreadths below the costal margin on inspiration. Liver biopsy revealed steatosis (planimetric determination showed that fat droplets constituted 5.4% of the section area)

Table VI. *Liver function tests and DF-induced urinary iron excretion in case 6 Compare with Fig 5*

	Upper limit of normal	Date (month, year) X, 1965	I, 1966	I, 1967	IX, 1967	IV 1968
SR (mg/100 ml)	1 1	0 5	0 3	0 3–0 9	0.5	0 4
Th (extinction)	0.10	0 01	0.01	0.02	0.01	0.01
AP (U)	8	8	8	7	5	6
SGOT (U)	40	44	40	25	43	20
SGPT (U.)	40	65–70	65	30–40	87	40
BSPR ( )	5	4–8	5	1 3	5	
Fe/urine (mg)	1.22	0.73	0.53	0 72	0 99	0 47
Fe/urine (µg/kg body wt)	14.3	9.4	6.8	9.0	12.4	5.9
UUP (µg/24 h)	200	2 800	2 360	445	6 900	530

Porphyrin analyses showed considerably increased UUP (7 800 µg/24 h) but UCP was normal (110 µg/24 h). Excretion of precursors was normal (ALA 2.4 mg, PBG 1.1 mg/24 h). Faecal porphyrins were increased (FCP 82, FPP 85 µg g dry wt).

Intravenous glucose tolerance was decreased ($k_g = 0.70$). Laboratory study of liver function showed slightly increased transaminase levels and BSPR was slightly increased (Table VI).

Hemoglobin concentration (14.7 g/100 ml) was normal. Iron studies showed normal serum iron (133 µg 100 ml), and the DF-test indicated iron stores of normal size (Table VI). Chemically determined non-heme liver iron concentration (80 mg/100 g dry wt, 1.3 mg 100 g liver protein content) was normal. The amount of hemosiderin in parenchymal liver cells (grade +) was not increased, but in Kupffer cells there was more iron pigment (grade +) than usually present in normal livers.

The phlebotomy treatment was started in the beginning of Nov 1965. As shown in Fig. 5 five phlebotomies were performed in Nov. and Dec. 1965. The hemoglobin level fell to 10.4 g 100 ml. Later the hemoglobin level and the serum iron increased. For this reason three more phlebotomies were carried out, which produced persistent anemia, sideropenia and low DF-induced urinary iron excretion. However significant amounts of iron were not mobilized from stores by the three latter phlebotomies, and iron available for hemoglobin synthesis was probably used up in Jan. 1966. The amount of iron that had been removed from stores was 0.5 g. In liver biopsy performed at the end of Jan. 1966 no hemosiderin could be detected.

The urinary porphyrin excretion was essentially unchanged during the first months of therapy but showed a decrease in Feb. 1966, and it decreased to level below 1 mg in the following month. It further diminished, and in Jan. 1967 porphyrin analyses gave the following results: UUP 445 µg, UCP 150 µg/24 h, FCP 5 and FPP 1 µg g dry wt.

Skin fragility gradually disappeared and he has had no skin symptoms since March 1966.

Laboratory tests indicated that liver function improved after therapy (Table VI). Intravenous glucose tolerance studied in Jan. 1967 also showed improvement (k_g = 0.94).

He was given 1 100 mg of iron dextran intravenously in Jan. 1967 (Fig. 5). The urinary porphyrin excretion remained at a low level during the following four months, but increased to high values during the period July to Sept. 1967 (UUP 800 to 6 900 µg 24 h). On examination in Sept. 1967 he had no skin symptoms and enjoyed good health. The serum iron was high (203 µg 100 ml), and the DF-induced urinary iron excretion was of the same order as before therapy (Fig. 5). The serum transaminase levels had increased (Table VI).

A new series of phlebotomies was performed from Sept. to Nov 1967. Iron stores available for hemoglobin production were depleted by the middle of November and amounted to 0.9 g of iron. In spite of anemia, sideropenia, and a low DF-induced urinary iron excretion and absence of sideroblasts there was reticular iron of grade + in bone marrow smears. Two more phlebotomies were performed, but no iron available for hemoglobin production could be mobilized from stores.

The urinary porphyrin excretion remained essentially unchanged during the first two months of phlebotomy therapy but then gradually decreased and fell to level below 1 mg/day in March 1968. It then further decreased, and in April 1969 the UUP was 200 µg/24 h. In April 1968 hepatic porphyrin fluorescence of grade + was present. Liver function tests again improved after therapy (Table VI).

DISCUSSION

A slight or moderate increase of iron stores is common in patients with porphyria cutanea tarda (14), and Ippen (6) tried phlebotomy therapy in this disease because of the similarities between PCT and hemochromatosis. However he was later reluctant to ascribe the effect of phlebotomy to the reduction of iron stores and suggested that the effect was due to the removal of porphyrins and porphyrin precursors (7). Also other authors considered it less probable that phlebotomy produces remission by reduction of iron stores (8, 9 16, 17). There are two main reasons for this. Firstly uroporphyrinuria is not ordinarily a feature of hemochromatosis. Secondly phlebotomy is effective not only in patients with overt iron overload but also in patients with quantitatively normal iron stores (4 10). Nevertheless, the present study indicates that there is a causal relationship between the presence of storage iron and the severity of the metabolic disturbance in PCT since in all five patients in whom new iron stores were built up after phlebotomy-induced remission a biochemical relapse ensued. In a previous study (10) 13 patients, who did not receive medical iron, were observed for periods of 1 to $3^1/_2$ years after phlebotomy induced remission and in eight of them the observation period after accomplished phlebotomy exceeded two years. In only one of these (who according to the desferrioxamine test had reaccumulated iron stores of considerable size) did a biochemical relapse occur. This took place after a remission period of two years.

The results of iron replenishment in case I should be of special interest, because an ideal control subject, viz. his twin brother (case) was available (Fig. 1). These twins, who are very much alike were studied by Waldenström and Haeger Aronsen, who performed blood group de-

terminations which indicated identity (21). At the start of phlebotomy case 2 had a more active disease than his brother who had very discrete skin symptoms. The urinary porphyrin excretion was somewhat higher in the former too. However, they had iron stores of the same size and their biochemical response to phlebotomy was very similar. The uroporphyrin excretion remained low in case during the observation period (3 $^1/_2$ years after phlebotomy). Also his desferrioxamine-induced iron excretion remained at a comparatively low level. In case 1, in whom iron stores were repleted by oral iron medication, the urinary porphyrin excretion showed increased values 1 $^1/_4$ year after the first course of phlebotomy.

The increase of the urinary porphyrin excretion was not closely related to the increase of the iron stores as measured by the desferrioxamine test. Thus in case 1 the desferrioxamine test indicated that new iron stores had reaccumulated after the first short course of iron medication (Fig. 1). An increased porphyrin excretion did not occur until $^1/_4$ year later when he had received oral iron medication for a prolonged period. Similarly in case 3 a desferrioxamine-induced urinary iron excretion of the size initially present was found in Nov. 1966. However, a significant rise in porphyrin excretion did not occur until 4 to 5 months later. Also in case 6, who was given iron dextrin intravenously, a significant increase of the urinary porphyrin excretion did not take place until several months later.

The fact that the urinary porphyrin excretion usually remained low for some months in spite of the DF test indicating a considerable reaccumulation of iron, is not a serious argument against the concept that storage iron has a causative role in this disease, but may have many explanations. The present type of porphyria is classified as hepatic because of the accumulation of porphyrins in the liver and absence of porphyrin excess in erythropoietic cells (18). It is believed that the disturbance of the porphyrin metabolism is confined to the liver cells. It seems probable that iron storage must take place in the hepatocytes to cause deterioration of porphyrin metabolism. Hence it is not surprising that it may take a considerable time until intravenously administered iron dextrin causes impairment of porphyrin metabolism, because such iron is initially phagocytosed by reticuloendothelial cells (2) and redistribution to parenchymal liver cells may require long time (2). Moreover, although the presence of storage iron in the liver seems to be a prerequisite for activity of PCT, there are many other factors of significance for the impairment of porphyrin metabolism, the most important one being alcohol consumption. The disease may improve in association with alcohol abstinence (10) and porphyrin excretion may even become normal (20). This indicates that the liver may tolerate larger amounts of storage iron without deterioration of liver porphyrin metabolism in porphyrics who decrease their alcohol consumption. This might explain why porphyrin excretion remained low for a considerable period in cases 3 and 4 who stopped alcohol consumption, and in case 1 who decreased his alcohol ingestion.

Another possible cause of a delayed increase in porphyrin excretion in patients with reaccumulated iron stores may be storage of porphyrins in the liver. Schmid et al. (18) showed that the liver may contain very large quantities of uroporphyrin in this porphyria. They obtained values up to 78 mg of porphyrins/100 g wet liver. It is conceivable that, with deterioration of porphyrin metabolism in PCT, large amounts of porphyrins may be stored in the liver before a significant increase in urinary porphyrin excretion takes place.

As pointed out before (10) phlebotomy is effective also in patients with quantitatively normal iron stores. This is illustrated in the present study because in three of the men liver non-heme iron concentration was determined before the initial course of phlebotomy and showed values of 62 (case 5), 80 (case 6) and 98 (case 4) mg/100 g dry wt. These values are below the mean (104 mg/100 g dry wt) but within the range (45 to 247 mg) of 20 control men (13). They also had moderate amounts of parenchymal liver cell hemosiderin (grade +, which was a common finding in controls (13)). The fact that phlebotomy produces remission also in patients with iron stores of normal size indicates that even ordinary amounts of storage iron may have a noxious influence on porphyrin metabolism in these patients.

In the second phlebotomy series storage iron of less than 1 g could be mobilized in two, in whom 0.6 (case 5) and 0.9 (case 6) g of iron was available for hemoglobin synthesis. This

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LIPID COMPOSITION OF HUMAN LIVER BIOPSY SPECIMENS

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Abstract. Fine needle biopsy specimens from patients with diseases accompanied by fatty liver have been analysed for triglycerides, phospholipids, free and esterified cholesterol. In selected cases the fatty acid composition of triglycerides was determined. The concentration of the lipids was not found to vary with the fundamental disease. A positive correlation between esterified cholesterol and triglycerides was verified in the presence of moderately but not of severely fatty liver. An association between esterified cholesterol and the surface area of fat droplets in the liver is suggested.

Several papers dealing with lipid analysis of needle biopsy specimens from human liver have recently been published (e.g. 5, 10). Only one study has included separate determination of free and esterified cholesterol in a material of obese and hyperlipidemic subjects (10).

The purpose of the present paper was to ascertain whether the distribution of the chief lipids in human fatty livers varies with the type of the fundamental disease. Elaboration of sensitive analytical methods enabled analysis of fine needle specimens with a dry weight of 0.080–2.34 mg (M = 1.030 mg).

MATERIAL

The clinical series consisted of 38 subjects, viz. three apparently healthy subjects, seven obese individuals, two with maturity onset diabetes mellitus (one of these 2 was studied on 3 occasions during treatment), 13 alcoholics, abstinent for 4 to 60 days, six patients with psoriasis, and three with porphyria cutanea tarda, diseases, which are frequently associated with fatty liver (1, 3) (2 of the 3 patients in the last group were studied before and after blood shedding), and four patients with pronounced fatty liver but without any other demonstrable disease.

METHODS

Biopsy specimens were usually obtained with a needle, 0.7 mm in diameter (13). In a few cases a Menghini needle, 1.4 mm in diameter was used. The aspirated specimen was washed in cold saline and stored at −30°C until analysed. The liver sample was transferred to an aluminium weighing pan and placed on an aluminium block (100 × 30 × 30 mm) precooled to −35°C. The specimen was afterwards freeze-dried for two hours in a desiccator over phosphorous pentoxide. Constant weight was obtained in half an hour. The specimen was then weighed on a Cahn gram electrobalance (range 10 mg) and homogenized in 2 ml of chloroform-methanol (2 : 1) in a Potter-Elvehjelm apparatus. The extract was filtered through a plug of glass yarn to a glass-stoppered centrifuge tube. Quantitative transfer was secured by washing three times with 1 ml of chloroform-methanol. The extract was purified by shaking with 1 ml of saline. The chloroform phase was evaporated in a stream of nitrogen and dissolved in 1 ml of heptane. 0.1 ml was used for semiquantitative evaluation of the triglyceride content by thin-layer chromatography (TLC) (8). Triolein in amounts of 5, 25, 50 and 150 μg was run simultaneously and the triglyceride was estimated by visual comparison after charring with sulphuric acid.

The remaining heptane extract was separated on a small silicic acid (BIO-SIL HA, Bio-Rad) column (diameter 3 mm, height 45 mm). A slurry of 300 mg of Biosil, freshly activated at 125°C for 12 h, was transferred to the column with the aid of a teflon catheter and was allowed to settle through a few cm of heptane in the glass column. Esterified cholesterol (ECH) was eluted with 6 ml of benzene-hexane (20 : 80, v/v), triglycerides (TG) with 4 ml of ether-hexane (5 : 95, v/v), free cholesterol (FCH) with 5 ml of chloroform, and phospholipids with 5 ml of methanol. The separated fractions were dried in a stream of nitrogen at 45°C.

The separation method was evaluated by separating extracts from serum analysed for FCH and ECH according to Sperry and Webb (12) with addition of glycerol tri(palmitate-1-^{14}C) (Amersham, England). At least 99% of the added ^{14}C-labelled tripalmitin was recovered in the TG-fraction, which contained less than 2% of the total cholesterol. The amounts of ECH and FCH, determined after chromatography corresponded to those expected with due allowance for the analytical errors. At least 95% of the lipid phosphorus was recovered in the phospholipid fraction.

The ECH fraction was dissolved in 5 ml, the FCH

Table I. *Lipid composition of human liver biopsy specimens*

Case 11 a, before treatment. b, after treatment for two months with tolbutamide. c, after another month with insulin.
Cases 33 and 34 a, before. b, about one year later after repeated blood shedding.

Case no	TG	ECH	FCH	PLP
	Lipids, g/100 g of dry liver			
	Normal			
1	1.6	0.18	1.24	12.9
2	2.0	0.19	0.90	8.5
3	3.5	0.23	1.24	6 9
	Obese			
4	10.5	0.40	1.96	8.5
5	17.2	0.49	1.21	8.6
6	16.5	0.31	1.32	8.0
7	18.2	0.46	1.30	6.9
8	26.3	1.00	2.00	8.6
9	26.8	0.28	0.81	6.8
10	37.3	0 48	1.33	5 4
	Diabetes			
11a	38.6	0.70	1.32	—
11b	20.9	0.61	2.58	7 6
11	17.5	0.55	1 68	8.0
12	22.5	0.48	1 43	7 6
	Alcoholics			
13	1.6	0.11	0.67	6.3
14	3.2	0.20	0.94	7.0
15	3 8	0.20	1 11	11 1
16	4.0	0.26	2.32	11.0
17	7.0	0 40	1.81	6.6
18	9.2	0.34	0 75	7 2
19	13 9	0.32	0.97	8.8
20	17.8	0.48	1 47	4 1
21	18.6	0 32	0.65	5.2
22	21.2	0 64	1 65	7 8
23	49.5	0.44	0.66	3.3
24	57.6	0.64	1.60	—
25	73.4	0 29	0.57	2.7
	Psoriasis			
26	10 4	0.34	1 19	9 4
27	10.7	0.36	1 18	6 2
28	17.0	0.40	1.68	9 4
29	31.4	0 36	0.74	6 0
30	41.0	0 60	3.12	6.5
31	60.7	0.56	1 45	5.3
	Porphyria cutanea tarda			
32	14 8	0.24	0.70	5 6
33	17 1	0 44	0 75	5..
33b	22.0	0.44	1.02	6.7
34	42.3	0.36	0.56	4.8
34b	47.0	0.36	0.58	—
	Unclear			
35	18.2	0.36	0.96	4.2
36	23 0	0.62	1.55	6.9
37	43 4	0.94	4.68	5.4
38	47 7	0.49	0.99	4.6
Mean	22.5	0 41	1.29	7 0

tion, in 10 ml of chloroform. Cholesterol was determined by slight modification of fluorometric method (15). all volumes were reduced to one fifth, and heating was performed in glass-stoppered centrifuge tubes for 30 min at 50°C Brief centrifugation was necessary to eliminate turbidity The standard curve was linear up to 4 μg of cholesterol. The coefficient of variation, calculated from double determinations, was 1.55%

TG were determined by slight modification of a method described earlier (6), based on fluorometric enzymatic glycerol analysis (7). TG were calculated in mg, assuming molecular weight of 885 Based on the results from TLC, portion of TG corresponding to about 10 μg was hydrolyzed in conical centrifuge tubes for 15 min t 55–60°C with 0.1 ml of freshly prepared mixture of 0.5 ml of 2 N KOH in water and 9.5 ml of ethanol. Fifty μl of 1.5 N acetic acid was then added. The sample was then evaporated in stream of nitrogen, and the last traces of acetic acid were removed in desiccator over KOH pellets. The residue was dissolved in 0.2 ml of water and the glycerol analyses were performed in the way described (7). This modification is suitable for 2 to 20 μg of TG The coefficient of variation, calculated from double determinations, was 1.93%

Methyl esters f fatty acids are prepared by refluxing in % H_2SO in methanol. Gas-liquid chromatography (GLC) f fatty acid methylesters was performed with a column of Gas Chrom P coated with 13 1% of ethylene g ycol succinate.

Lipid phosphorus was determined and converted to phospholipids by multiplication by 25 (2).

Statistical treatment was performed in computer according to the BMD-03R program (4)

RESULTS AND DISCUSSION

In the present investigation the analytical results are given relative to dry liver weight for two

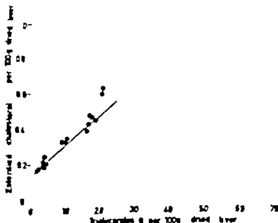


Fig 1 Relationship between esterified cholesterol and triglycerides in human liver The regression line $y = 0.016 + 0.159X$ valid for TG ≤ 25 g per 100 g dried liver is drawn.

reasons. The samples were regularly fragmented, and blotting of the small pieces would have been very difficult. The weight gain of the dry samples after removal from the desiccator was of the order 1.5% during the first minute, thus very small, compared with the weight loss of moist samples. The second reason was that it was much easier to homogenize freeze-dried samples. For comparison with results expressed as per cent of wet weight, a factor of 3.2 may be used assuming the mean water content of normal human liver to be 69% (9), although underestimation may result at high fat content.

The analytical results are given in Table I and Figs. 1 2 and 3 There was no systematic variation in the relative amounts of different lipids with the basic diseases, possibly with the exception of case 37 (fatty liver of unknown cause) FCH was highest in this case.

Despite the use of quite different methods, our results are mainly in agreement with those obtained by Reumanen et al. (10) in their obese and hyperlipidemic subjects. However some differences were noted, which might depend on the dissimilarities in the composition of the materials. Thus, only ten of Reumanen et al.'s (10) 37 subjects had TG values exceeding 16 g per 100 g of dry liver compared with 29 of our 4 samples.

Reumanen et al. (10) found a significant correlation between ECH and TG (=0 70) so did we, but with $r = 0.47$ Judging from Fig. 1 the correlation was probably based on samples with TG $\leq$ 25 g per 100 g of dry liver The material

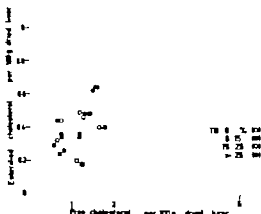


Fig 2. Relationship between esterified and free cholesterol in human liver (% = g per 100 g dried liver).

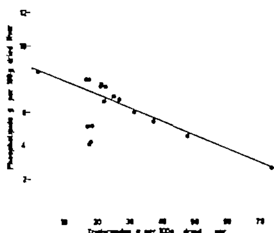


Fig 3 Relationship between phospholipids and triglycerides in human liver Regression line $r = -0.081X + 8.7$

was therefore divided, somewhat arbitrarily at this TG level. A significant correlation, =0.88, was found between ECH and TG for subjects with TG $\leq$ 25 g per 100 g of dry liver (n = 78), but not for those with higher TG levels (n = 14) = −0.21

On the other hand, the correlation between ECH and FCH was but little dependent on the TG concentration =0.67 for samples with triglycerides $\leq$ 25 g per 100 g of dry liver as against 0 76 for samples with higher triglyceride values. For the lower level of TG the multiple correlation coefficient for the relation between ECH, CH, and TG was as high as 0.91 The regression may be formulated as ECH = 0.035 + 0.108 (S.D 0 0 4) FCH + 0.0149 (S.D 0 0016) TG In our material the correlation between FCH and TG was not significant (= 0.08).

Experiments with cholesterol-fed rats (11) have shown that most of the increase in the cholesterol of the liver depends on the ester fraction. On ultracentrifugation the ECH was concentrated in floating fat. As floating fat is composed essentially of TG the association between TG and ECH in our investigation is not surprising. However the increase of ECH with TG is not a simple function of solubility as the correlation disappears *at very high TG levels.*

It is tempting to suggest that the amount of ECH is function of the total surface of the fat droplets. With increasing amounts f TG these

Table II. *Composition of the main fatty acids in liver TG*

Case no	Diagnosis	TG (g/100 g dry liver)	Fatty acids, % of total						
			14:0	15:0	16:0	16:1	18:0	18:1	18:2
1	Normal	1.40	3.7	1.7	37.8	7.9	12.1	28.2	7.7
2	Normal	2.0	1.3	0.9	27.8	6.6	14.0	38.0	11.4
6	Obese	16.5	1.3	—	32.1	12.7	5.5	41.2	7.3
11	Diabetes	38.6	2.0	1.5	38.7	16.8	5.0	31.0	5.0
16	Alcoholic	4.0	5.2	1.6	39.6	9.2	8.1	27.9	8.4
24	Alkoholic	57.6	4.7	1.1	46.5	11.2	5.2	27.6	3.6
29	Psoriasis	31.4	1.4	0.6	33.6	10.9	9.1	38.7	5.0
32	Porph. cut. t.	14.8	2.2	1.3	35.1	9.1	4.1	39.5	8.6
35b	Porph. cut. t.	22.0	2.0	0.7	24.4	8.0	6.6	41.4	15.0
34b	Porph. cut. t.	47.0	0.7	0.3	38.8	9.6	4.8	37.1	8.7
37	Unclear[a]	45.4	4.0	1.0	35.6	10.1	4.7	35.6	9.0
38	Unclear[a]	47.7	2.0	—	42.8	9.8	3.9	36.3	5.2

Porph. cut. t., porphyria cutanea tarda.
[a] Fatty liver without any other disease.

droplets tend to coalesce, with the formation of fewer and larger fat vacuoles. This process is accompanied by a reduction of their total surface area. In accordance with this hypothesis, the fatty vacuoles tended to be smaller in cases with high ECH compared with those with low ECH but similar TG contents.

The phospholipid level was found to vary inversely with TG ($r = -0.64$). This correlation disappeared when phospholipids were expressed as g per 100 g of TG free liver. This may be regarded as a manifestation of a low phospholipid content in the fat droplets compared with the other parts of a liver cell with massive fat infiltration. The absence of such a correlation in the investigation by Kremer et al. (5) may be due to differences in the materials.

The fatty acid compositions of liver TG from selected cases analysed with GLC are presented in Table II. No clear variation was found with the type of underlying diseases. That the stearic acid level tended to be lower and the palmitoleic acid higher with increasing TG is in accordance with the observations of Takahashi and Tanaka (14) in three cases of fatty liver. The somewhat high level of myristic acid in the two alcoholics may be representative, although our material is not large enough to warrant any conclusions. The low linoleic acid value in case 24 was probably due to deficient intake, as he had been a heavy drinker for several years.

ACKNOWLEDGEMENT

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Acta med. scand. Vol 189 pp. 69–72, 1971

ELEVATION OF URIC ACID CLEARANCE CAUSED BY INAPPROPRIATE ANTIDIURETIC HORMONE SECRETION

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Abstract. Two patients with hyponatraemia resulting from an inappropriate ADH secretion syndrome had low serum values and elevated clearances of uric acid. All the values returned to normal after fluid restriction. The same changes in uric acid clearance and serum level were seen when this syndrome was experimentally induced in five normal subjects.

Abnormally low serum uric acid levels, most often resulting from increased renal clearance, are sometimes encountered in renal tubular disorders (9), in normal pregnancy (1) and in an occasional patient with liver disease (4). The pathogenesis of these conditions, in which the balance between reabsorption and secretion of uric acid by the renal tubules is apparently changed, is unknown.

The finding of low serum uric acid levels in two patients with hyponatraemia, probably resulting from inappropriate antidiuretic hormone (ADH) secretion, prompted us to explore the reason for this abnormal renal behaviour. Since the syndrome of overhydration can easily be reproduced in normal subjects, information could be gained concerning the physiological influences on uric acid excretion by the kidney.

MATERIAL

In patients A and B, men aged 76 and 65 respectively, low serum levels of Na, Cl and uric acid were discovered accidentally when they presented with vague complaints. An asymptomatic but inoperable lung carcinoma was present in patient A, while no diagnosis could be made in patient B. Creatinine and urea clearances were normal. Water diuresis was completely abolished in the presence of urine osmolalities superior to 520 and 478 mosm/l, respectively, after drinking water

METHODS

Creatinine and electrolytes were determined by the autoanalyzer technique, and uric acid by the uricase method of Praetorius (6). Plasma and extracellular fluid volumes were measured by means of J 131 labelled human albumin and the Br 82 dilution method (5). The subjects were on normal salt intake, while purine-rich food was withheld.

In five subjects, three women and two men, water retention was caused by three injections of five units Pitressin® Tannate in Oil at 15 to 18 h intervals, followed by water ingestion so as to maintain body weight 3–5% above control level during –4 days.

RESULTS

Observations on the two patients

Since uric acid clearance may vary considerably in one person, determinations were made during several days both over 24 hour and over shorter periods.

The determinations were repeated after a few days of fluid restriction, during which the body weight had decreased by 1.3 and 2.[illegible] kg, respectively.

The results are shown in Table I. Serum uric acid was abnormally low and its clearance elevated during the time that serum sodium was depressed. All values returned to normal range after fluid restriction. In patient A, body fluid volumes were also measured.

Experiments in normal subjects

In the five normal subjects, serum uric acid diminished after water ingestion and Pitressin® injection, roughly parallel to serum Na levels, and remained low as long as the body fluid expansion was maintained.

Table II summarizes the data. In Fig. 1 the

Table I. *Influence of fluid restriction in the two patients*

	Patient A		Patient B	
	Control	After 1 week of fluid restr.	Control	After 4 days of fluid restr.
Serum N conc. (mEq/l)	129	138	125	144
Serum uric acid (mg/l)	21	46	27	54
Creat. clearance (ml/min)	129	104	97	84
Uric acid clearance (ml/min)	20.0	7.5	18.2	8.1
Body weight (kg)	75.3	74	65.5	63.3
Br⁸² volume (l)	19.4	18.6	—	—
Plasma volume (l)	3.2	3.0	—	—

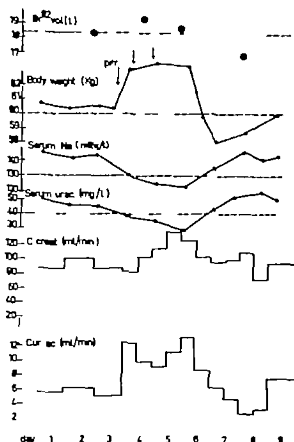


Fig. 1. Subject no. 1. Influence of water drinking and simultaneous inhibition of water diuresis on several variables.

full sequence of events in a representative experiment is given. In most of the subjects the creatinine clearance rose, as it did in the one represented in the graph, but to a lesser degree. The ratio of uric acid to creatinine clearance was always elevated during the Pitressin®-water loading period. After cessation of the ADH activity a "rebound" occurred, during which the body weight fell below the original value and marked Na retention occurred. At the same time, uric acid clearance fell below control values. The data on the five experiments are summarized in Fig. 2.

In two of the normal subjects, Br 82 distribution volume and plasma volume were measured before, during and after the experiment. The results are given in Table III.

DISCUSSION

To our knowledge no data have been published on uric acid clearance in the "inappropriate ADH secretion" syndrome. High uric acid clearances were reported by Weinstein et al. (11) in two patients with lung carcinoma, who also had (slightly) elevated amino-acid excretion. The authors ascribed these abnormalities to some toxic tubular damage, but in retrospect these patients

Table II. *Effect of overhydration in normal subjects*

Subject no.	1		2		3		4		5	
Day of body fluid expansion	Before	3	Before		Before	3	Before	2	Before	3
Serum Na conc. (mEq/l)	146	126	140	126	141	130	138	130	141	128
Serum uric acid conc. (mg/l)	50	28	50	24	36	20	41	21	49	29
Uric acid clearance (ml/min)	5.8	13.6	7.4	19.1	11.2	4.0	11.4	25.5	7.8	12.2
Creatinine clearance (ml/min)	92	118	106	117	138	151	135	127	98	104
Body weight (kg)	60.3	63.1	57.4	60.1	68.4	70.0	71.8	73.9	67.5	70.0

may have suffered from the same syndrome as ours. In one of them, serum sodium values of 132 and 117 mEq/l are reported. Our observations suggest that elevation of uric acid clearance can be related to the expansion of the extracellular compartment, which occurred spontaneously in the patients and resulted from the simultaneous water loading in the test subjects. This expansion is generally held to be responsible for the "paradoxical" Na excretion in this condition (12). The Br 82 volume was indeed found to be elevated although not very much. The excess diminished from +1.3 l to +0.5 l in subject 5 and even from +0.8 l to 0.1 l in subject 1 during prolonged expansion experiments.

The results are consistent with the observations of Cannon et al. (2), who found a large rise in uric acid clearance after NaCl infusions.

On the other hand, sodium depletion and extracellular fluid volume contraction, whether produced by thiazide diuretics or not, have been shown by Suki et al. (10) to result in a depression

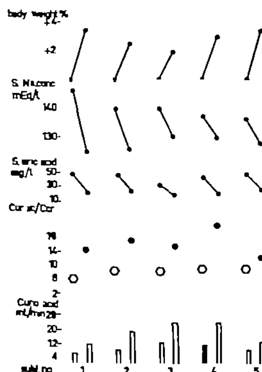


Fig. 2. Summary of the water loading experiments. Two values from each subject, one on the day before Pitressin® injections started, the other on second or third day of expansion. Clearance values and clearance ratios are the mean values during these same days.

Table III. *Changes in body fluid compartments during two experiments, Pitressin injections on day 4 and 5*

Day no.	3	5	6	8
Subject 1				
Body weight (kg)	60.3	63.1	63.2	58.7
Br^{82} volume (l)	18.3	19.1	18.4	16.5
Plasma volume (l)	3.0	—	3.1	2.9
Subject 5				
Body weight (kg)	67.5	70.1	70.0	67.8
Br^{82} volume (l)	19.6	20.9	20.1	19.0
Plasma volume (l)	3.6	—	3.7	3.5

of uric acid clearance and a rise in serum uric acid levels. The observations both of Cannon et al. and of Suki et al. have been confirmed by us (unpublished data). It seems likely therefore, that the regulatory mechanisms which stimulate the kidney to Na excretion and Na retention, in order to maintain extracellular fluid volume stability exert a similarly directed influence on the renal uric acid clearance. Whether this change in clearance is a result of changes in tubular reabsorption or secretion cannot be judged from our experiments. In view of the supposed near complete reabsorption of filtered uric acid (3), changes in secretion rate seem more likely. Steele (8), using pyrazinamide suppression of urate secretion, reported that expansion and contraction of extracellular fluid caused significant decrease and increase, respectively in urate reabsorption. However in absolute terms these changes were not large enough to account for the changes in clearance observed by Suki et al. (10), Cannon et al. (2), and in the present study. Moreover Steele (7) could not reproduce the increase in uric acid clearance after saline infusion observed by Cannon et al. This may be related to difference in duration of the experiments, if a longer period of expansion (as in our experiment) is required to bring about the full effect on uric acid clearance.

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THE LIPOPROTEIN PATTERN IN A DANISH FAMILY

PRELIMINARY REPORT

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Abstract. The lipoprotein pattern in a family of 14 members with primary hyperlipidaemia has been studied on the basis of clinical examination and the Fredrickson-Lees criteria for biochemical investigation of lipoproteins. The patterns were of the following distribution: type II (2), type III (2), type IV (7), and new types with the prefix normo- suggested by the authors in family studies —normo-type II (2) and normo-type IV (1). In spite of the biochemical findings, 11 of the probands had none of the clinical symptoms known to characterize lipoproteinaemia. Finally the authors discuss the relationship between pattern typing by paper electrophoresis, by ultracentrifugation, and by immunoelectrophoresis using the Laurell method.

The purpose of this preliminary study was to investigate the lipoprotein pattern in 14 members of the same family by means of lipoprotein electrophoresis and analytical ultracentrifugation. Concurrently a clinical assessment was performed.

METHODS

The study was carried out in the autumn of 1969 on 13 siblings and their father. Two probands, who lived close by were examined in the hospital, while all the others were visited in their homes. In the clinical assessment a complete history was taken with a view to symptoms and signs known to belong to the clinical features of hyperlipoproteinaemia (Hlp)—and in particular to ruling out diseases which might give rise to secondary Hlp—and the patient had a physical examination including determination of the blood pressure, ophthalmoscopy and ECG. All the probands were closely questioned concerning medication (especially with hormones), smoking and drinking habits. In several cases supplementary information was gained by borrowing case records from hospitals or obtained from general practitioners.

All the probands were investigated at 8 a.m. after fasting overnight for 14 h. None had been on dietetic treatment.

All samples were transported to the laboratory in a refrigerating box (about 10°C to avoid denaturation of the lipoprotein complex due to fluctuations in temperature), and all the lipid analyses were started 6 hours after removal of the samples. With the exception of the serum lipid electrophoresis all the analyses were completed on the same day.

Samples for immunoelectrophoresis by the Laurell (7) method and ultracentrifugation were forwarded by express delivery. The first ones were examined by H. G. Nielsen, Med. Labs, Copenhagen, about 14 h after the sampling, and the last ones by K. Erdal, Carlsberg Breweries, Copenhagen, at the time that the laboratory found best suited.

Serum

Total lipid (TL): Chabrol and Charonnat, with the modification of Zöllner and Kirsch (17).

Total cholesterol (TC): method of Huang et al. (6).

Triglyceride (TG): UV test, Biochemica-test, Boehringer Mannheim, W. Germany.

Lipid-phosphorus (LP): phosphorus determined as molybdenum complex.

Total beta-lipoprotein (TbL): after protein precipitation the total cholesterol in the precipitate was determined and the TbL calculated by multiplying by a factor of 2.63.

Lipoprotein electrophoresis: method of Lees and Hatch (8).

RESULTS

The distribution by age, sex, height, weight, percentage of overweight, and occupation is presented in Table I. Table II gives clinical data that are not included in the case histories. Most of the probands were small, of pyknic build, with a

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Table I. *Clinical features*

No.	Sex	Age	Height (cm)	Weight (kg)	n-weight[a] (kg)	±	Occupation
1	♂	47	155	61	56	+10	Manufacturer
2	♂	45	172	85	68	+25	Labourer
3	♂	43[b]	160	79	60	−35	Labourer
4	♂	43[b]	166	81	64	−25	Contractor
5	♀	42	154	58	53	−10	Housewife
6	♂	39	160	76	60	−30	Labourer
7	♂	37	164	68	63	−10	Upholsterer
8		35	168	70	64	−10	Domestic help
9		34	156	60	56	−10	Grocer
10	[illegible]	37[c]	166	69	64	−10	Clerk
11	♂	30	157	67	59	−15	Electrician
12		28	162	58	60	− 5	Grocer
13		25	1[illegible]8	63	58	−10	Factory worker
14	[illegible]	71	165	75	63	−20	Former roadman
(15		68[d]	154	63	55	+15	Housewife)

[a] Normal weight after Natvig (12). [b] Dizygotic twins. [c] Father. [d] Mother (deceased).

varying degree of overweight. In spite of this constitution, nearly all had mild neurasthenic symptoms.

There was no family or personal history of diabetes mellitus, myxoedema, pancreatitis, hepatic or renal diseases.

Cutaneous or tendinous xanthomas were not present or had been present in any proband, and there had been no instance of hepato-splenomegaly. For corneal arcus, cf. Table II.

The following measurements were normal. Hb, ESR, Hct., PBI, T_3-test, GOT, alkaline phos-

Table II. *Clinical features*

No.	Xanthoma	Corneal arcus	Ophthalmoscopy	BP	ECG	Vascular disease
1	—	—	Normal	160 100	Iso-T_{I} [illegible]	Intermittent claudication, central and peripheral neurological symptoms
2	—	—	Normal	135 90	Left axis deviation	—
3	—	—	Arterio-sclerot. angiopathy	160 85	Normal	Mild angina pectoris
4	—	—	Normal	125 85	RBBB[a]	—
5	—	—	Normal	150 80	Iso-T_{III}	—
6	—	—	Normal	150 95	Low voltage	Incipient intermittent claudication
7	—	—	Normal	120 60	Normal	—
8	—	—	Normal	135 85	Normal	—
9	—	—	Normal	120 80	Normal	—
10	—	—	Normal	140 80	Normal	—
11	—	—	Normal	130 85	Normal	Embolism of popliteal artery, intermittent claudication, angina pectoris, transient cerebral ischaemia
12	—	—	Normal	120 60	Normal	—
13	—		Normal	130 85	Normal	—
14	—	—	Normal	150 75	Left axis deviation	—
15[b]	Unknown	Unknown	Hypert. fundus I°	200 130	Q_{III}, T_{III} (infarct)	Coronary sclerosis with infarct, hypertension. Autopsy: generalized arterio-sclerosis

[a] Right bundle-branch block. [b] Mother (deceased).

Table III *Biochemical features*

Pat. no.	Type	TL	TC	TG	LP	TbL	Serum
1	IV	1 060	263	212	10.2	620	Clear /cloudy
2	III	2 110	300	920	15.1		Creamy
3	IV	1 440	306	404	11.9	692	Cloudy
4	n–IV	1 070	282	179	10.2	564	Clear
5	IV	1 180	242	328	11.5	573	Clear/cloudy
6	IV	1 585	313	520	13.2	684	Cloudy
7	IV	1 390	319	419	13.1	710	Clear/cloudy
8	II	1 060	295	119	12.2	615	Clear
9	n–II	982	268	104	9.8	476	Clear
10	III	1 170	280	343	13.2	705	Clear
11	IV	1 318	335	360	12.8	648	Cloudy
12	n–II	728	188	108	8.2	375	Clear
13	II	1 095	295	145	11.3	594	Clear
14	IV	1 112	270	242	10.3	590	Clear/cloudy

Reference range after fasting for 14 hours
Total lipid (TL) < 1 000 mg/100 ml.
Total cholesterol (TC): 140–300 mg/100 ml. Conversion factor to mmol/l. 259 10^{-4}
Triglyceride (TG): 74–172 mg/100 ml. Conversion factor to mmol/l 114 10^{-4}
Lipid-phosphorus (LP). 6.4–10 6 mg/100 ml. Conversion factor to mmol/l. 323 10^{-4} and to phospholipids mg 100 ml. 23.5
Total betalipoprotein (TbL): 550 mg/100 ml. Conversion factor to mmol/l. as TC.
Too creamy to be analysed by the method used.

phatase, thymol turbidity serum paper electrophoresis, bilirubin, blood glucose (overnight fasting) and NEFA (= FFA) in all patients. The urine microscopy and Hema-Combistix® were normal too.

The results of the biochemical investigations are given in Tables III–V

CASE REPORTS

Case 1

At the age of 30–40, uncharacteristic epigastric and precordial pain, dyspnoea, palpitations, and dizziness. These complaints were interpreted as cardiac neurosis and nicotine poisoning during stay in hospital in 1957 while during another stay in 1959 they were thought to be gastritis. X-rays of the stomach showed no signs f ulcer. ECG and chest radiography normal. Glucose tolerance test normal. About the age of 40, intermittent claudication. In 1968 admitted to the Department of Vascular Surgery Hospital of Odense. Angiography showed arteriosclerotic changes reaching from the abdominal aorta to the distal part of the lower-limb arteries. In the common iliac artery stenosis, 1 cm in length, treated by by-pass operation. The arteriosclerotic changes were confirmed at operation. The walking distance changed from 90 m 85 sec to 500 m/450 sec, and the patient was relieved of symptoms. Creatinine, BP and ECG normal. Chest radiography aortic arteriosclerosis. In 1969 transient paraesthesias and exertional pain in the right hand and arm, blurred vision, and diffuse headache. Good pulsation th radial artery but the arm as rather cyanosed and cool. The physical examination was otherwise normal.

Case

Always in good health. Physical examination normal.

Case 3

Occasionally precordial pain, but otherwise in good health. Physical examination normal

Case 4

Appendicectomy in 1940. In 1960 acute appendicitis, confirmed by exploratory laparotomy In 1961 physical therapy for osteoarthritis of the spine, and in 1964 ectomy Previous and present physical examination normal.

Case 5

Appendicectomy in 1943 In 1953 bed ridden at home, and in 1967 admitted because of fatigue headache and increased need of sleep N organic cause could be demonstrated. Hi neurasthenic complaints were unchanged. Physical amination normal

C e 6

In 1951 bronchopneumonia. For some years atypical angina pectoris A tendency to orthostatic oedema without varices For about ten years restless and painful right leg W lking ability about 1 000 m, followed by resting for 10 15 min. Examination revealed acrocyanosis and ... of lividity on elevation. Good peripheral pulsation Other physical findings normal.

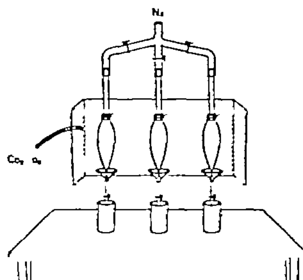


Fig. 1. Apparatus for production of ultrafiltrate.

redistilled water and fixed to the plastic-covered ground-glass joints of [illegible] glass tube. Adhering water was sucked out of the membrane before the serum specimen was poured in and covered with paraffin. The collodium membrane was then placed in [illegible] transparent polyethylene box (Fig. 1), through which was directed [illegible] saturated CO_2-O_2 mixture with [illegible] pCO_2 corresponding to the ear capillary blood [illegible] f the patient. After equilibration for half an hour the ultrafiltration was started under nitrogen pressure. The procedure lasted for [illegible] 3 hours, and three specimens with identical pCO_2 could be ultrafiltrated at the same time. The filtrate was collected through [illegible] hole in the bottom of the box, after throwing away the first 1 ml, as the concentration of calcium was thereafter constant.

Calcium was determined in serum and ultrafiltrate by the EDTA murexide method (22); phosphorus by the method of Fiske and Subbarow (6) modified by Müller (13). Coefficient of variation 0.6% and 3% respectively. The coefficient of variation for ultrafiltrable calcium was 0.8% for ultrafiltrable phosphorus [illegible]1% determined during the following days.

Ionized calcium was determined by the method of Rose (16), modified by Hahnemann (9), all determinations were made in duplicate, coefficient of variation for the double determinations 0.9%, day to day reproducibility 0.9%. Samples were drawn on the two following days from 17 patients, and the mean values are given. The coefficient of variation for the concentration of ionized calcium determined in this way was found to be [illegible]0%. Complex-bound calcium was determined as the difference between ultrafiltrable and ionized calcium.

The sodium concentration was determined on serum and ultrafiltrate by flame photometry (coefficient of variation 1.2%) for the purpose of calculating the Donnan effect and as a control of the procedure of ultrafiltration.

Inulin and 3-day endogen creatinine clearance was determined to evaluate the function of the kidneys. Inulin analyses were performed by the method of Bojsen (4), creatinine was determined on an autoanalyzer (Technicon), coefficient of variation 3.6%.

Creatinine clearance was determined in all patients. Inulin clearance was also determined in ten of the uraemic patients. In these ten patients there was no significant difference between inulin and creatinine clearance. For the calculations the creatinine clearance was used.

Total protein was determined by refractometry, coefficient of variation 3.5 [illegible], albumin by agar gel electrophoresis, cutting out the coloured spot and eluating the dye-stuff with subsequent spectrophotometry. Serum alkaline phosphatase was determined by dephosphorylation of p-nitrophenylphosphate (2), coefficient of variation 6%. In nine patients the analyses were repeated at a later date.

RESULTS

1. *Calcium*

In the statistical evaluation of the material all values of serum concentration of calcium, total phosphorus and total sodium were corrected for serum water by multiplication by 1000 (990–8

Table I. *Concentration of calcium and phosphorus fractions in serum in uraemic patients and in normal persons*

	Uraemia: Clearance 20 ml/min, n=20	Clearance 20 ml/min, n=10	All uraemics, 30	Normals, 20
Total calcium (mg/100 ml)	9.38±1.01	9.91±0.43	9.57±0.89	10.62±0.33
Ionized calcium (mg/100 ml)	6.11±0.63	6.40±0.26	6.21±0.54	6.45±0.26
Complex-bound calcium (mg/100 ml)	0.71±0.[illegible]1	0.54±0.26	0.65±0.25	0.90±0.39
Protein-bound calcium (mg/100 ml)	[illegible]56±0.48	[illegible]97±0.[illegible]4	2.71±0.45	3.27±0.45
Ionized calcium in % of total calcium	65.[illegible]±2.[illegible]	64.6±2.5	64.9±2.5	60.8±3.5
Total inorganic phosphorus (mg/100 ml)	5.96±[illegible]29	3.83±0.71	5.20±2.13	3.93±0.37
Ultrafiltrable phosphorus (mg/100 ml)	5.35±[illegible]04	3.32±0.66	4.63±1.43	3.40±0.37
Ultrafiltrable phosphorus in % of total phosphorus			88.9±6.7	86.9±4.6

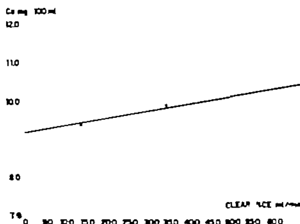


Fig. 2. Correlation between the concentration of total calcium and clearance. r = +0.347 0.05 > p > 0.02.

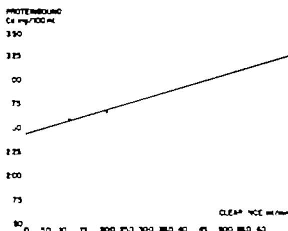


Fig. 4. Correlation between the concentration of protein-bound calcium and clearance. r = +0.431 0.01 > p > 0.001

(prot.)), prot. being the serum concentration of proteins in g/100 ml (18). The concentration of ultrafiltrable and ionized calcium was corrected for Donnan effect by multiplying the values obtained by Na-Sw/Na-uf, Na-Sw being the serum concentration of sodium corrected for serum water and Na-uf the concentration of sodium in the ultrafiltrate (16). The concentration of ultrafiltrable phosphorus was corrected for Donnan effect by multiplying the values determined by 0.915 (21). In normal persons the values of serum calcium were 10.62 ± 0.33 mg%, ionized calcium 6.45 ± 0.26 mg%, protein-bound calcium 3.27 ± 0.45 mg%, complex-bound calcium 0.90 ± 0.39 mg% (Table I). Comparing these values with those of the uraemic patients (Table I), the mean values of the latter group were significantly lower ($t = 7.36$, 2.14, 4.57 and 2.84 for total calcium, ionized calcium, protein-bound and complex bound calcium, $p < 0.001$, $0.05 > p > 0.02$, $p < 0.001$ and $0.01 > p > 0.001$). The relation ionized calcium/total calcium was higher in the uraemic patients than in the normal persons (0.65 against 0.61, $t = 4.73$, $p < 0.001$).

Comparing the values for the uraemic patients with a creatinine clearance below and above 20 ml/min, the concentration of total calcium, ionized calcium and protein-bound calcium was significantly lower in the patients with severe urae-

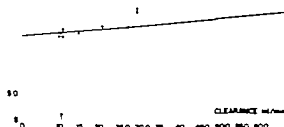


Fig. 3. Correlation between the concentration of ionized calcium and clearance. r = +0.221 p > 0.1



Fig. 5. Correlation between the concentration of complex-bound calcium and clearance. r = +0.03 p > 0.1

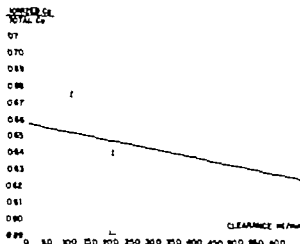


Fig. 6. Correlation between the relation ionized calcium/total calcium and clearance. $r=+0.324$, $0.05>p>0.02$.

min ($t=2.30$, $0.05>p>0.02$; $t=2.07$, $0.05>p>0.02$; $t=11.4$, $p<0.001$).

The relationship between creatinine and total calcium is registered in Fig. 2, between clearance and ionized calcium in Fig. 3, between clearance and protein-bound calcium in Fig. 4 and finally between clearance and complex-bound calcium in Fig. 5. We found a positive correlation as far as total calcium and protein-bound calcium were concerned, while there was no significant correlation between clearance and ionized and complex bound calcium, respectively. We found a slightly negative correlation between clearance and the relation ionized calcium/total calcium (Fig. 6).

There was no correlation between the concentration of complex-bound calcium and serum citric acid ($r=-0.199$, $p>0.1$), nor between serum albumin concentration and protein-bound calcium ($r=0.144$, $p>0.1$) even though the serum albumin concentration was reduced in the uraemic patients (mean value 3.37 ± 0.57 mg/100 ml, normals 4.10 ± 0.40 mg/100 ml). The calcium-binding ability of serum protein is expressed by the formula $K_{Ca\ prot.}=\text{Kuf}\times(\text{prot-pk})/\text{pk}$, Kuf being ultrafiltrable calcium, pk protein-bound calcium, both measured in mol per l serum, prot. is the serum concentration of protein in mol per l calculated as $1.22\times$ total protein concentration in g/100 ml (15, 21). The calcium binding ability for patients with clearance <20 ml/min was 0.0119 mol ±0.0056, and for patients with clearance >20 ml/min $0.0088\pm0.00?7$, both significantly different from mean values in the normal adults ($M=0.0050\pm0.0014$, $t=5.9$ and 4.8, $p<0.001$).

We could not demonstrate any correlation between the calcium fractions and pH, nor between ionized calcium/total calcium and pH, and there was no difference in the relation in patients with acidosis (pH<7.35) as compared to the other patients (Fig. 7).

2. *Phosphorus*

The serum concentration of phosphorus was negatively correlated to clearance (Fig. 8), but it appears that only patients with clearance <20 ml/min had increased values. Ultrafiltrable phosphorus constituted 86.9 ± 4.6% of total serum phosphorus in the normal persons against 88.9 ± 6.7% in the uraemic patients, and the difference was not significant. There was no correlation between the relation ultrafiltrable phosphorus/total phosphorus and clearance ($r=-0.22$, $p>0.1$), nor between pH and the same relation ($r=0.08$, p

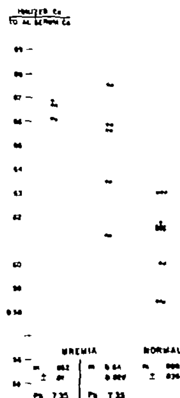


Fig. 7. The relation ionized calcium/total calcium in serum in uraemic patients with a pH determined on capillary blood below and above 7.35 and in normal persons.

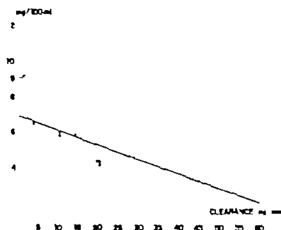


Fig. 8. Correlation between the concentration of total inorganic phosphorus and clearance. $r = -0.602$ $p < 0.001$

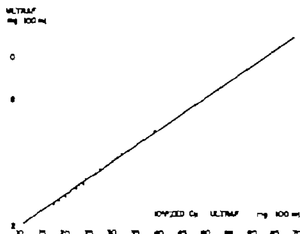


Fig. 10. Correlation between the concentration of ionized calcium × ultrafiltrable phosphorus and the concentration of ultrafiltrable phosphorus. $r = +0.971$ $p < 0.001$

>0.1) Nor was the relation correlated to the concentration of total calcium or total phosphorus ($r = -0.134$ and $r = +0.015$ $p > 0.1$)

3. *The calcium × phosphorus product*

The product of the concentration of ionized calcium and ultrafiltrable phosphorus was in normal persons 21.98 ± 2.44 while the value for the uraemic patients was 28.54 ± 11.66. The product was negatively correlated to clearance (Fig. 9). There was no correlation to pH ($r = -0.02$, $p > 0.1$) nor to the concentration of ionized calcium ($r = 0.19$ and 0.20 $p > 0.1$) (Fig. 11).

Eighteen of the 30 uraemic patients exhibited radiological changes of the bones in the form of halisteresis, and in one patient spontaneous fractures. Bone changes were demonstrated in 12 of 20 patients with clearance > 20 ml/min $= 60$% and in 6 of 10 patients with clearance < 20 ml/min $= 60$%. In 14 of these patients the serum concentration of alkaline phosphatase was increased. Three of the 12 patients without radiological bone changes had elevated values of alkaline phosphatase. Mean values for alkaline phosphatase in patients with and without bone changes were 3.07 ± 1.11 units/100 ml and 1.89 ± 0.18 units/100 ml, respectively. The difference was significant ($t = 3.6$, $0.01 > p > 0.001$). Calcium

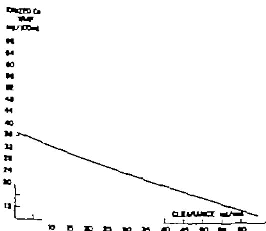


Fig. 9. Correlation between the concentration of ionized calcium × ultrafiltrable phosphorus and clearance. $r = -0.584$ $p < 0.001$.

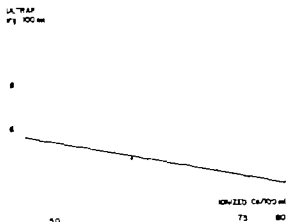


Fig. 11. Correlation between the concentration of ionized calcium and the concentration of ultrafiltrable phosphorus. $r = -0.190$ $p > 0.1$

Table II. *Concentrations of serum calcium and serum phosphorus fractions in uraemic patients with normal and increased concentration of serum alkaline phosphatase*

	Serum alkaline phosphatase	
	Normal concentration (<3.2 U) −13	Increased concentration (>3.2 U) −17
Total calcium (mg/100 ml)	10.01±0.76	9.24±0.86
Ionized calcium (mg/100 ml)	4.42±0.52	4.04±0.53
Complex-bound calcium (mg/100 ml)	0.69±0.30	0.61±0.19
Protein-bound calcium (mg/100 ml)	2.89±0.36	2.57±0.47
Ultrafiltrable phosphorus	4.50±2.01	4.63±1.92

and phosphorus fractions in patients with normal and with elevated values of alkaline phosphatase are shown in Table II. It appears that all calcium fractions tended to be lower in patients with increased values of alkaline phosphatase, while the concentration of ultrafiltrable phosphorus was identical in the two groups.

DISCUSSION AND CONCLUSION

Several authors (3 5 21) have emphasized the fact that the calcium fractions are differently proportioned in uraemic patients than in normal persons, as the protein-bound calcium is relatively lower whereas the complex bound fraction is relatively higher Our experiences are in agreement with this fact, as far as patients with severe uraemia are concerned, even though a significant correlation was demonstrated only between protein-bound calcium and clearance, while there was no significant negative correlation for the complex-bound fraction. On the other hand, we found a negative correlation between the relation ionized calcium/total calcium and clearance, in spite of the fact that ionized calcium was reduced in patients with clearance <20 ml/min This is contrary to the findings of Walzer (21) and Better et al. (3). Regarding ultrafiltrable phosphorus, our experiences were in agreement with Walzer's (20), who found that the relation ultrafiltrable phosphorus/total inorganic phosphorus was unchanged in uraemic patients as compared to normal persons.

We did not find any reverse correlation between serum calcium and serum phosphorus in uraemic patients, in accordance with Stanbury and Lumb (19) but in contrast to Better et al. (3) who demonstrated such a correlation in conformity with relations in normals and in hypoparathyroid patients. The ionized calcium ultrafiltrable phosphorus product increased with decreasing renal function this is due to the fact *that the product was correlated to ultrafiltrable* phosphorus.

Regarding serum proteins, we found that the calcium-binding ability was reduced in the uraemic patients, an observation also reported by Walzer (21) in contrast to Better et al (3).

In this material we found no relation between pH and ionized calcium which is in accordance with the investigations of Walzer (21) and Better et al (3) However Albright et al. (1) and Peterson et al. (15) are of the opinion that acidosis increased the serum concentration of ionized calcium.

The demonstrated disagreement between our findings and those of other investigators may be explicable by the differences in the procedure of ultrafiltration and preservation. Walzer (21) indicates that the samples are kept deep-frozen which may alter the concentration of ionized calcium. In both of the before-mentioned investigations the ultrafiltration was undertaken at a constant pCO_2 in this work, however it was carried out at a pCO_2 corresponding to that of the patient.

The fact that ultrafiltrable phosphorus constitutes approximately 90% of the concentration of total inorganic phosphorus may indicate that patients with severe renal failure, in whom phosphate clearance (determined on the basis of total serum phosphorus) approaches creatinine clearance, would in fact have a tubular secretion of phosphate if the calculations were based on ultrafiltrable phosphorus instead of total inorganic phosphorus. Phosphate clearance would hereby be increased by approximately 10% and in some cases, presumably turn out to be higher than creatinine or inulin clearance.

As already stated, the ionized calcium ultrafiltrable phosphorus product was well correlated to clearance and this correlation was mainly caused by the elevation of serum phosphorus in increasing uraemia. The cause of the reduction of ionized calcium in patients with severe uraemia

has been the subject of much discussion. As a possible cause is often mentioned the elevation of inorganic phosphorus in these patients which increases the product and may cause metastatic calcifications and tend to decrease the concentration of ionized calcium (19). Metastatic calcifications could not be demonstrated in our material —neither for patients as a whole, nor for patients with clearance below 20 ml/min, nor for patients with a duration of disease exceeding two years.

The previous statements concerning the relation between the serum concentration of calcium and phosphorus in uraemia has been very variable. Stanbury and Lumb (19) demonstrated a positive correlation between total calcium and phosphorus. All these patients had renal bone lesions. Better et al. (3) found a reverse correlation between ionized calcium and ultrafiltrable phosphorus, none of the patients in their material had major bone alterations. We (8) could not demonstrate any significant reverse correlation between total calcium and phosphorus in 16 untreated uraemic patients, 10 of which had changes of the bones.

Previous studies of the frequency of bone lesions in patients with uraemia are scanty and the reason is presumably that the evaluation of bone biopsies and X ray studies is difficult. In our material only two of the patients had major radiological changes of the bones. In total 18 of the patients had radiological bone lesions (halisteresis) 12 of whom suffered from severe kidney insufficiency. The number of patients with bone lesions in the group with severe and with moderate renal insufficiency was the same.

Comparing the radiological bone lesions with the elevation of serum alkaline phosphatase, we found that the elevation was significantly higher in patients with bone lesions, which is in accordance with a previous paper (8). If the calcium fractions and phosphate concentration in patients with normal and elevated serum alkaline phosphatase are examined, it appears from Table II that patients with elevated serum alkaline phosphatase tend towards lower concentration of all calcium fractions, while the concentration of ultrafiltrable phosphorus is identical in the two groups. These findings indicate to some extent that approximately half of a group of uraemic patients seems to have light, and in some cases severe, radiological changes of the bones, that these changes occurred as frequently in patients with moderate uraemia as in patients with severe uraemia, and that there seems to be some relation between the serum level of alkaline phosphatase and radiological changes of the bones. On the whole, patients with elevated alkaline phosphatases had lower calcium fractions, but the same values of ultrafiltrable phosphorus as patients without these alterations. In this connection it is worth noting that exchangeable calcium, calcium turnover and mineral accretion rate evaluated by Ca^{47} technique tend to be increased in these patients (8).

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PERMANENT ENDOCARDIAL PACING

Seven Years' Experience

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Abstract. Forty-nine patients have been treated with permanent pacing, 48 of them with endocardial electrode. *In four patients the pacing was begun with epicardial* electrodes, which were later substituted by endocardial electrodes in three of them. Altogether 101 impulse generators were implanted. The early postoperative mortality was 2% (1/48). The cumulative survival rates at one, two and three years were 90, 69 and 63% respectively. The battery life-time was 15.2 months on the average. The commonest complication was dislocation of the endocardial electrode, which happened in five patients as an early complication and in three patients later during the follow-up period. This complication was treated successfully in all instances. The advantages of endocardial in comparison to epicardial electrode technique are discussed.

The endocardial electrode to be used in pacemaker treatment of chronic heart block was introduced by Lagergren and Johansson (17). Since then several reports on this method have been published (3, 7, 8, 11, 12, 15, 18, 29, 30). The major advantage of this method is the relatively simple procedure during which only local anesthesia is needed.

The endocardial electrode technique has been used exclusively in our hospital since 1963. The following report covers our experiences of this method as well as those based on a few early cases in which the treatment was begun with epicardial electrodes.

MATERIAL AND METHODS

Between 1961 and 1969, 49 patients have been treated by permanent pacemaking in our hospital. The indications for pacemaker implantation have been strict, the indication being Adams–Stokes syndrome in 94% of the cases (46/49). The mean age of the patients was 65.7 years, ranging from 31 to 86 years. There were 25 males and 4 females.

The duration of symptoms before implantation of pacemaker is seen in Table I.

The etiology of the underlying heart lesion could not be specified in 29 patients. Coronary heart disease was present in 13 patients, rheumatic heart disease in six, and congenital heart disease in one patient. Cardiac failure was present in 22 patients.

The usual ECG finding was complete AV-block, being present in 34 cases. Intermittent AV-conduction disturbances were seen in 12 patients. Two patients presented atrial fibrillation and slow ventricular response and one patient had sinus arrest with slow idioventricular rhythm.

During the years 1961 and 1962 four patients were treated with epicardial pacemakers implanted by means of thoracotomy. Both the epicardial electrodes and the batteries were manufactured by Elema-Schönander, Sweden. In three of these patients the epicardial electrodes were later substituted by endocardial.

Since Jan. 1963 the endocardial unipolar electrode (Elema–Schönander) technique requiring only local anesthesia has been used exclusively. The electrode has been inserted through the external jugular vein in all except four instances, in which the internal jugular vein was used. The pulse generator has been implanted in the rectus muscle sheet in 93 cases, subcutaneously (abdominal wall) in five, and elsewhere in three cases.

Altogether 101 pulse generators have been implanted. The commonest model used has been fixed rate Elema-Schönander battery (EM 137, 139, 142, 152), 87 instances. Since 1968 an increasing number of ventricular triggered pacemakers (EM 153) have been implanted. During last year four Medtronic Inc. (Chardack-Greatbatch 5870 C) batteries with unipolar Elema electrodes have been implanted.

The routine controls of patients were made at 1, 4, 7 and 10 months after pacemaker implantation, and then every month. Before the detailed test system described below pacemaker failure was detected by clinical symptoms, ECG and pacemaker rate change.

Since April 1969 all patients were tested immediately after implantation and then in routine controls by recording the pacemaker-induced skin potentials on a fast-

Table I. *Duration of symptoms before implantation of pacemaker*

Time	No of pats.
< 1 month	5
1–6 months	21
6–12 months	3
> 12 months	20

Table II. *Survival rate after implantation of the first pacemaker*

Years since operation	No. of pats.	Alive	(%)
> 5	8	3	(38)
4–5	1	1	(—)
3–4	10	5	(50)
3	7	5	(72)
1 2	12	9	(75)
< 1	11	11	(100)

moving cathode-ray oscilloscope (Tektronix 502 A), using ordinary ECG limb electrodes. The impulse was photographed by Polaroid camera for exact measurements of maximum amplitude, amplitude at 0.5 ms (decay ratio), and duration. Criteria for impending pacemaker failure were as follows: fall in maximum amplitude 20% or more, decay ratio change 20% or more, duration change 10% more, rate change 5 impulses min or more (2, 9 23 25 28).

RESULTS

Survival rate

Of the total of 49 patients 34 are still alive.

One patient, aged 70 years, died in myocardial infarction a few hours after the operation. The early mortality was thus 2% (1/49).

The survival rate after the implantation of the first pacemaker is given in Table II.

The cumulative survival rate is seen in Fig. 1 One patient with congenital heart block and severe Adams–Stokes syndrome is still alive eight years after the initial battery implantation. This is the only long-term survival case among those with initially epicardial electrodes. In this patient the electrodes were detached and subsequently substituted by endocardial electrodes in 1965

Duration of battery function

Emergency change refers to cases when the battery was exhausted, causing acute symptoms which necessitated immediate therapy and implantation of a new pacemaker battery (Table III). The commonest finding in connection with emergency changes was bradycardia, and in some cases Adams–Stokes attacks.

Elective change means that the procedure was undertaken before the patients developed clinical symptoms pointing to impending battery failure.

The battery has often been changed in connection with electrode failure in spite of a perfectly working pulse generator The estimated life-time has in these cases been such as to warrant the change. Also in cases with infection the battery has been removed prematurely and a new one installed.

COMPLICATIONS

In 32 out of 49 patients no complications related to pacemaker implantation were seen.

Epicardial electrodes (4 patients)

In two patients the batteries had to be removed because of infection. These patients died 23 months and 36 months after the implantation of the first pacemaker, respectively In the third patient infection caused detachment of the epicardial electrodes. This patient was subjected to altogehter 15 operations, including three thoracotomies, until she died in purulent pericarditis 31 months after the first operation. In the fourth pa-

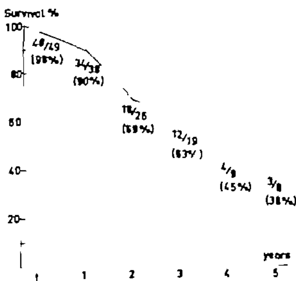


Fig. 1 Cumulative survival rate after start of pacing.

tient the detachment of epicardial electrodes necessitated the change to endocardial.

In three of these four patients the epicardial electrodes were substituted by intravenous endocardial electrodes.

Endocardial electrodes (48 patients)

Complications related to pacemaker apparatus or its implantation (battery failure excluded) occurred in 14 patients.

A number of complications were clearly related to the endocardial electrode technique (Table IV). Early complications could be treated by exposing the electrode with an incision made in the subclavian fossa and pushing the electrode back to the right ventricle. The late complications often necessitated insertion of a new endocardial electrode.

Six patients had complications which could be related to surgical procedures (Table V). Infection in the battery pocket" could in all cases be treated by removing the battery and the infected portion of the electrode. In addition, new battery had to be implanted in a new place.

DISCUSSION

The indications for long-term pacemaker treatment have been rather strict in our hospital and an attempt has been made to treat the suitable cases conservatively before pacemaker implantation. This accords well with the report by Redwood (22), who emphasises the favourable results obtained with conservative treatment in selected patients with chronic heart block. The survival rates at one, two and three years were 76% 64% and 57% respectively. In patients in whom treatment by pacing became necessary in order to control symptoms, the survival rates were 83% 72% and 60% respectively. On the other hand the one-year survival rate in an unselected patient group with chronic heart block has been reported to be as low as 50% (14).

The primary mortality in our material was low % and corresponds well with figures given by several authors, who also used the endocardial electrode technique (3 4 6, 7 12). The primary mortality is lower than that for epicardial electrodes, which in several series has been in the range of 3 to 15% (5 6, 7 13, 15 19 4, 29). The more complicated surgical technique requir

Table III. *Battery life and cause of battery change*

Cause of battery change	No. of batteries	Average life-time (mo. (range))
Battery failure		
Emergency change	27	15.5 (1 29)
Elective change	15	14.5 (11 18)
Total	42	15 2 (1 29)
Electrode failure	8	10.5 (5–22)
Infection	8	8 9 (1 17)

Table IV *Failures related to endocardial electrode*

Failures	No. of instances	Treatment
Early complications (6 pats.)		
Electrode dislocation		Reposition of electrode
Right atrium	3	
Pulmonary artery	1	
Coronary sinus	1	
Diaphragmal twitching	1	Reposition of electrode
Late complications (8 pats.)		
Increased threshold (?)	4	Insertion of new endocardial electrode
Displacement into right atrium	3	Reposition into right ventricle (2 cases), new electrode (1 case)
Electrode in coronary sinus	1	New electrode
Wire break	1	Extension lie

Table V *Complications related to surgery in endocardial pacing (6 patients)*

Complications	No. of instances	Treatment
Postoperative ound infection	3	Battery change (2 cases) Battery change and new endocardial electrode (1 case)
Postoperative ound hematoma	2	Evacuation and resuturation
Necrosis of overlying skin		
Extrusion of endocardial electrode	3	Resuturation
Extrusion of indifferent electrode plate	2	Resuturation
Battery extrusion	1	Battery change

ing general anesthesia and thoracotomy is apparently responsible for the rather high primary mortality when using epicardial electrodes.

The survival rate during the follow-up period was in our material almost similar to those reported previously (1 3 10 11 12, 16, 19 20 21 27) The long-term results of permanent pacing seem to be similar in patients treated either by endocardial or epicardial electrode technique.

The battery life-time in our series was 15.2 months, which corresponds well with the experiences of e.g., Gotsman et al. (8) Grendahl (9), Storch et al. (26) and Tala et al. (29) with the same impulse generator Elema–Schönander The battery life-time is to be regarded as too short, as the resulting need for battery changes requires a considerable number of surgical procedures which are harmful to the patient and carry the risk of infection.

The number of patients who had been admitted to hospital because the impulse generator had become exhausted, and in whom an emergency change of the battery had taken place, was remarkably high. This reflects the difficulties which have been encountered in estimating the remaining charge of the battery in routine controls of the patients, before the criteria now in use had been established. The value of the pacemaker impulse test now in routine use in our laboratory cannot yet be estimated, but it is expected to reduce the need for emergency change of a worn-out impulse generator Especially the increased impulse duration seems to be a useful indicator of impending failure of Elema–Schönander pacemakers (9 23 28).

The dislocation of the endocardial electrode out of the right ventricle is a well-known drawback of this technique (3 7 10, 16 26, 30) In our series this happened five times in the immediate postoperative period, and three times as a late complication. This complication is easily detected and treated during the first postoperative days while the patient is still hospitalized. If it happens later the consequences may be more serious as the pacing ceases suddenly In our series the three patients with this late complication survived until the endocardial electrode was replaced. However a similar late complication, the electrodes getting detached from the myocardium (8 16) is occasionally seen also when the epicardial electrode technique has been used.

Myocardial perforation by the endocardial electrode has been described by several authors (1 4 6 12, 26). In our materal this complication was not seen, which apparently is due to the soft Elema–Schönander endocardial catheter used in all cases. Wire breaks, which have been numerous in many other series (4 5 6 19 29), were also rare when this catheter was used.

The complications related to surgery were in our material relatively few as compared to figures given in connection with epicardial pacemakers and thoracotomy (12, 15 20) In our material surgical complications were seen in six out of 48 patients, or 12.5% all of whom could be treated successfully without discontinuation of pacemaker treatment.

There are still divided opinions about the relative superiority of epicardial and endocardial pacing in long-term treatment. A great number of favourable reports on the endocardial technique have been published (3 6 7 8, 11 12, 18 29), although in some centres epicardial pacing is still preferred and the endocardial technique is used only in aged and debilitated patients (13 15 30) Since 1963 the endocardial electrode technique has been applied in our hospital to every patient requiring long-term cardiac pacing and we have not come across a single case in which epicardial pacing would have been preferable.

In our experience long-term endocardial pacing has no particular disadvantages as compared to the epicardial technique. In addition, the primary mortality is low and the long-term results compare well with those obtained by epicardial pacing.

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INDIVIDUAL PLASMA PHOSPHOLIPIDS IN HYPERCHOLESTEROLEMIA

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Abstract. Individual phospholipids were determined in the plasma of 24 hypercholesterolemic subjects and in 13 healthy controls. In the hypercholesterolemic group total phospholipids were increased, as are the absolute amounts of lecithin and especially sphingomyelin. Expressed as percentage of total phospholipids, sphingomyelin was increased and lecithin decreased. The absolute amount of lysolecithin was unchanged and the percentage lower than normal. Phosphatidylethanolamine was not changed. The findings are compatible with an increase of beta-lipoproteins of normal composition in hypercholesterolemia. It is speculated whether the alteration in the phospholipid pattern may be of importance for the tendency to atherosclerosis in hypercholesterolemia.

The error of lipid metabolism which seems most atherogenic is the increase of beta-lipoproteins (hyperlipoproteinemia type II (6)), characterized by increased levels of cholesterol in blood but nearly normal triglyceride values. Intense studies have been performed on various aspects of this common and dangerous metabolic error. So far the interest in the plasma phospholipids has been slight, although total phospholipids have often been noted to be increased.

As the plasma phospholipids constitute a heterogenous fraction, composed of different compounds, it was considered to be of interest to investigate individual plasma phospholipids in a material of hypercholesterolemic persons.

MATERIAL AND METHODS

Plasma samples were obtained from 24 patients with hypercholesterolemia (cholesterol more than 300 mg/100 ml) but ithout hypertriglyceridemia (triglycerides less than 65 mM/l). There were 12 men and 12 women, mean age 54.0 years (range 40–71 years). They were selected from in- and out-patients at the Medical Department ho routinely had blood lipid examinations. Some were asymptomatic but most of them had symptoms of cardiovascular disease, e.g. angina pectoris or intermittent claudication. T men had diabetes, treated with oral antidiabetic drugs. None had thyroid disease. Some of the patients were taking drugs for their cardiovascular disease, e.g. digitalis, nitroglycerin or dicoumarol, but none was taking any drugs used to treat hyperlipemic states, e.g. nicotinic acid or clofibrate. Some of the patients had previously been advised to reduce their intake of calories and especially of fats but no patient as on rigid diet. None had had recent myocardial infarction.

The controls were selected from previously published material of healthy subjects (1). Persons below the age of 40 and with cholesterol more than 300 mg/100 ml ere excluded, and there thus remained 13 persons (6 men and 7 omen) of mean age 50.8 years (range 40–60 years).

Heparinized plasma as obtained in the fasting subjects, and total and free cholesterol, triglycerides and total and individual phospholipids ere analyzed as previously described (1).

Statistical analyses ere performed according to Snedecor (17).

RESULTS

Mean values and standard errors of the means are presented in Table I. Individual phospholipids are given both as the percentage of total phospholipids and as absolute amounts.

There is a statistically significant difference between the groups for both total and free cholesterol. Triglyceride levels are also higher in the hypercholesterolemic group, and this applies both when differences are calculated directly and after conversion to logarithms (*).

Total phospholipids are significantly higher in the hypercholesterolemic persons, and this is due to an increase of lecithin and especially of sphingomyelin, which is proportionately more increased than lecithin. Thus the percentage of sphingomyelin is increased and that of lecithin decreased. The amounts of lysolecithin and phosphatidylethanolamine are not changed, and consequently

Table I. *Plasma lipids in hypercholesterolemic persons and in a control group*

TC = total cholesterol. FC = free cholesterol. TG = triglycerides. PL = phospholipids. PE = phosphatidylethanolamine. Lec = lecithin. Sph = sphingomyelin. LL = lysolecithin. S.E.M. = standard error of the mean. *P* = significance of the differences between the two groups

		TC (mg/dl)	FC (mg/dl)	TG (mM/l)	PL (mM/l)	% of total PL PE	Lec	Sph	LL	mM/l PE	Lec	Sph	LL
Hypercholesterolemia = 24	Mean	406	125	1.76	4.51	1.4	68.1	25.2	5.2	0.06	3.07	1.14	0.23
	S.E.M	21.5	10.1	0.10	0.20	0.15	0.50	0.53	0.23	0.007	0.13	0.06	0.012
			(= 23)										
Control subjects = 13	Mean	224	65	1.03	3.36	1.5	70.7	21.5	6.3	0.05	2.38	0.72	0.21
	S.E.M	10.7	5.2	0.13	0.16	0.16	0.63	0.52	0.33	0.007	0.12	0.04	0.009
			(= 9)										
	P	< 0.001	< 0.01	< 0.001	< 0.001	N.S.	< 0.01	< 0.001	< 0.01	N.S.	< 0.01	< 0.001	N.S.

the percentages of these phospholipids are decreased. The decrease is statistically significant in the case of lysolecithin.

DISCUSSION

There is no widely accepted upper limit for normal cholesterol levels, and in large materials there seems to be a continuous increase from "normal" to pathological levels. For the purpose of this study an arbitrary upper limit of 300 mg/100 ml was defined. This necessitated the exclusion of some persons from our previously published "normal" material.

The upper limit of triglyceride levels is also not well defined. However Fredrickson et al. (6) state that a slight increase of triglycerides is not incompatible with the diagnosis of type II hyperlipoproteinemia, which is due to an increase of beta-lipoproteins. Lipid electrophoresis was not done in our material but we presume that our patients mainly belong to this type of hyperlipoproteinemia, although a slight increase of prebeta lipoproteins cannot be excluded.

Although it is well known that total phospholipids are increased in hypercholesterolemia, very few data have been published on the composition of the phospholipid fraction. Christian et al. (4) reported on a child with familial hypercholesterolemia. All the phospholipids were increased with the exception of lysolecithin, which remained normal in absolute amounts. Relatively sphingomyelin had increased most. Vikrot (22) reported on the phospholipid composition in two cases of hereditary hypercholesterolemia. There were no pronounced changes but the percentage of sphingomyelin seems to be high.

Martini et al (9) reported on the individual phospholipids in 30 cases of hypercholesterolemia due to various causes. Triglyceride values were not given and possibly other hyperlipoproteinemias than type II were included. The authors found a decrease of lecithin and an increase of lysolecithin, but no definite change of sphingomyelin or cephalin. The analyses were performed on serum, which may partly explain their findings, as transformation of lecithin to lysolecithin occurs even at room temperature (21). In addition, alcohol-ether was used for extraction of lipids while other investigators have used methanol-chloroform.

Gjone and Norum (7) presented data on individual phospholipids from five patients with primary hyperbetalipoproteinemia and found no definite changes in the phospholipid pattern. A study of their tables shows, however that the percentage of sphingomyelin was usually at the upper limit and that of lysolecithin at the lower limit of their normal range.

In the present investigation a rather characteristic change in the phospholipid pattern is evident. Sphingomyelin increased relatively most, so the percentage of this phospholipid was significantly increased.

Lecithin was also increased in absolute amounts but relatively less so and consequently its percentage was slightly lower than normal. The absolute amounts of lysolecithin and phosphatidyl-

ethanolamine were not changed. In the case of lysolecithin this entailed a significantly decreased percentage, while the change of phosphatidyl ethanolamine percentage was not significant, which may be due to methodological difficulties in the determination of this small fraction.

The general opinion seems to be that in isolated hypercholesterolemia there is an increase of beta-lipoproteins of normal composition (5 6 16). Thus the composition of isolated beta-lipoproteins from hypercholesterolemic persons has shown a normal pattern with respect to cholesterol, triglycerides and total phospholipids. To the best of our knowledge individual phospholipids have not been determined in separated lipoproteins from hypercholesterolemic persons, except by Smith (15). With the help of paper chromatography she found widely varying sphingomyelin and lecithin concentrations in low-density and high-density lipoproteins, so that no certain conclusions can be drawn.

Our findings are compatible with an increase of normal beta-lipoproteins (in the ultracentrifuge the beta-lipoproteins correspond to the low-density lipoproteins, isolated between densities 1.006–1063 or S_f subclass of 0–20). Steele and Kayden (18) presented evidence that the ratio of sphingomyelin to lecithin was higher in the beta-lipoproteins than in whole serum. In ultracentrifuge studies Phillips (11) determined the phospholipid composition of separated lipoprotein fractions and noted that the molar ratio of sphingomyelin to lecithin in the lipoproteins with density less than 1.063 was twice the corresponding values in the high-density lipoproteins. The lysolecithin was concentrated in the heavier fraction.

Nelson and Freeman (10) found the highest percentage of sphingomyelin in the S_f 0–20 fraction. They analysed phospholipids by infrared spectrometry by which method lecithin and lysolecithin were determined together. Goodman and Shiratori (8) found in one subject the percentage of sphingomyelin in lipoproteins of density 1 019–1.063 to be about double that in lighter or heavier lipoproteins.

Switzer and Eder (19) noted, as had previously been found by Phillips (12), that the major portion of the plasma lysolecithin is transported together with proteins other than the lipoproteins and is found in a fraction with density more than 1.21 They considered that lysolecithin was transported by albumin and they questioned whether the small amounts sometimes found in various lipoprotein fractions were not artefacts. Skipski et al. (14), in ultracentrifuge studies on serum found the highest sphingomyelin content in the LDL_1 class of lipoproteins with density 1.006–1 0635 and the highest concentration of lysolecithin in the ultracentrifugal residue.

The percentage of lecithin in various lipoprotein fractions has varied inversely with the sphingomyelin percentage in the preceding investigations. The cephalin fractions, where they have been measured, have been about equally present in all fractions.

To judge from these reports, an isolated increase of beta-lipoproteins would tend to give an absolute and relative increase of sphingomyelin, and an absolute increase but a lowered percentage of lecithin. Lysolecithin should be found in normal absolute amounts, but the percentage should be lower due to the increase of the other phospholipids. This is in accord with the findings in our study

Is the alteration in the phospholipid pattern of importance for the tendency to atherosclerosis which is so pronounced in hyperbetalipoproteinemia? At present no answer can be given, but it should be noted that in atheromatous plaques sphingomyelin is the predominant phospholipid (3). It seems to be at least in part synthesized locally as judged by incubation experiments with various radioactive substrates (3 20). The incorporation of labeled material into sphingomyelin is, however less than the incorporation into the other phospholipids. It is thus possible that a certain proportion of the sphingomyelin in the vessel walls may be derived from plasma and thus from the beta-lipoproteins Portman and Alexander (13) have presented evidence that, in nutritionally induced atherosclerosis in monkeys, the intimal uptake of sphingomyelin from plasma as well as the local synthesis is increased.

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Acta med. scand. Vol. 189 pp. 97–100, 1971

ADDITIONAL EVIDENCE FOR CHROMOSOME ABNORMALITIES IN THE ERYTHROID PRECURSORS IN ACUTE LEUKAEMIA

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Abstract. In continuation of a previous study five additional patients with acute leukaemia have been studied, characterized by abnormal bone marrow karyotypes and fairly well represented erythropoiesis. In all five patients the percentage of erythroblast mitoses (16–62% of the bone marrow mitoses) was definitely higher than that of normal metaphases. These data confirm the previous results and suggest that, in acute myeloid leukaemia, the chromosome abnormalities are not confined to the cells of the blastic variety but are also present in the erythroid precursors. Thus acute myeloid leukaemia is probably not a disease of the granulocytic cell line, but rather a disorder of haemopoiesis in general.

In a previous cytogenetic study of acute leukaemia (8) we reported on four patients with abnormal stem lines and numerous erythroblasts in the bone marrow in whom the prevalence of erythroid mitotic figures exceeded that of normal karyotypes. This was taken as evidence that the chromosome abnormalities were not restricted to the white blast cells, but were present also in the erythroid cell line. Since then, similar findings have been obtained in five additional patients with acute leukaemia. These studies are reported here.

MATERIAL AND METHODS

Subsequent to our previous report we have made karyotype studies on 26 additional patients with acute leukaemia. In five of these patients the chromosome number of bone marrow cells showed abnormal modes, and at the same time at least 10% of the mitotic figures of the bone marrow smears belonged to the erythroid series. The cytological types of the leukaemias are shown in Table I. Erythropoiesis was megaloblastic in cases III and IV and normoblastic in the remaining cases.

For cytogenetic study the bone marrow aspirates were treated according to the method of Tjio and Whang (12) without in vitro culture; the cells were exposed to colcemide for one hour. A metaphase was considered acceptable either if the chromosome number could be determined or if an abnormal marker chromosome could be recognized. The proportion of bone marrow mitoses belonging to the erythrocytic and granulocytic precursors was determined in Giemsa-stained smears (prepared immediately after aspiration) of the same samples as were used for cytogenetic study. Mitoses were classified as erythroid only if the mitotic cells corresponded in size and tinctorial characteristics to proerythroblasts, basophilic or polychromatic erythroblasts. All other mitoses were considered as non-erythroid. The differential count of mitotic figures was based on 100 mitoses in each patient.

RESULTS

Table II shows the results of the karyotype studies. Two patients had hyperdiploid stem lines, one had a hypodiploid line, and in two cases pseudodiploid stem lines were found. In case II a marker chromosome (a minute acentric fragment) was present in 26 of 27 metaphases.

In Table III the percentage of erythroid mitoses in the direct bone marrow smears is compared to the percentage of normal diploid metaphases in the chromosome preparations from the same marrow aspirates. From 16 to 6[illegible]% of the mitotic figures in the direct bone marrow smears belonged to the red cell series. The frequency of normal metaphases ranged from 0 to 22%. Considering the data on the individual patients, it is evident that in all patients the proportion of erythroblastic mitoses clearly exceeded the proportion of normal metaphases.

DISCUSSION

The cases of acute myeloid leukaemia [illegible] its variants (acute erythroleukaemia [illegible]

Table I. *Cytological type of leukaemia and therapy*

Pat. no.	Sex	Age (y)	Type of leukaemia	Chemotherapy prior to study
I	♂	70	Myeloblastic	None
II	♂	73	Myeloblastic	None
III		54	Acute erythro-leukaemia	None
IV	♂	44	Acute erythro-leukaemia	None
V	♂	65	Promyelocytic	None

Table III. *Comparison between the proportion of erythroid mitoses in the direct marrow smears and the proportion of normal metaphases in the chromosome preparations*

Pat. no.	Erythroid mitoses (%)	Normal metaphases (%)
I	16	4
II	18	4
III	52	0
IV	82	22
V	34	10

cytic leukaemia), which are the subject of this report, were characterized by a relatively good preservation of the erythroid cell line in the bone marrow. If erythropoiesis in these cases were the remnant of normal erythropoiesis, one would expect a prevalence of normal karyotypes corresponding to that of erythroid mitoses. The data show the contrary: the frequency of normal karyotypes does not account for the frequency of erythroid mitoses, and the data thus strongly suggest that in AML and its variants (acute erythroleukaemia and promyelocytic leukaemia) the chromosome abnormalities are not confined to the cells of the blastic variety but are present also in the erythroid precursors. This in turn would mean that, at least in part, erythroblasts in AML are not normal but part of the leukaemic cell line. However, it must not be overlooked that the evidence is indirect, since it is based on comparisons between chromosome preparations and direct marrow smears, albeit from the same aspirates. When interpreting the present data, some possible sources of error must therefore be considered.

1. Conceivably, erythroid mitoses might not spread as well as granulocytic mitoses, consequently they would be underrepresented among the scorable metaphases. In that case one should find a correlation between the number of non-scorable metaphases in the chromosome preparations and the ratio erythroid mitoses/total mitoses in the direct smears. As in a previous study (8), there is no such correlation (Table IV), which militates against the possibility raised.

2. Another possible source of error would be that erythroid mitoses might be more easily damaged than granulocytic mitoses during the processing for karyotype analysis, and hence underrepresented in the chromosome preparations. However, this is not likely, because damaged metaphases or single scattered chromosomes were not seen more frequently in material with numerous erythroblasts than with fewer erythroid mitoses.

3. The time from marrow aspiration to fixation of the chromosome preparations was one hour. If blast cells had a shorter mitotic time than normal marrow cells, the 1 hour colcemide block in vitro would lead to a higher relative frequency of blast cell mitotic figures in the chromosome preparations than in the direct marrow smears; the available evidence indicates that, on the contrary, leukaemic blast cells have a longer

Table II. *Chromosome findings in bone marrow cells*

Pat. no.	Total metaphases counted	Chromosome number <41	41	42	43	44	45	46	46[a]	47	No. of cells with marker chromosomes
I	50					1				47	
II	77					1			23	1	26
III	4	3		1		1	8				
IV	50							11	39		
V	50						5			45	

[a] Pseudodiploid cells.

mitotic duration than normal cells (4 5) and hence should rather be relatively underrepresented in the chromosome preparations.

4 A matter of greater concern to the interpre tation f the present data is that quite a substantial fraction of the metaphase figures in the chromosome preparations could not be evaluated, because of chromosome stickiness and indistinct chromosome morphology As shown in Table IV the scorable metaphases varied between _1 and 52%. It is universally recognized that in most leukaemic marrow aspirates the chromosomes of metaphases belonging to the abnormal stem lines are sticky and have a blurred and ill-defined appearance, which makes their scoring difficult (1 6, 7 11). This actually makes it likely that th majority of the non-scorable metaphases in th present series were abnormal, viz. aneuploid or pseudodiploid. However rather than relying on this, one may do the opposite and make the rather unlikely assumption that all the non-scorable metaphases were indeed normal diploid. Even so, the data still indicate that chromosome abnor malities are present in erythroblasts. In Table V the non-scorable metaphases and the observed diploid metaphases have been pooled under the heading maximal possibility of normal metaphases the table also includes data on the cases we have reported previously (8). It will be seen that in three cases (nos. 1 4 and 5) the percentage of erythroid mitoses clearly and in one case (no. III) slightly exceeds this maximal possibility of normal metaphases

The present results thus support our previous conclusion (8) that the karyotypically abnormal clones in AML and its variants are not confined to the white blast cells, but are present also in erythropoiesis. Therefore AML is not disease of the granulocytic cell line but rather a disorder of haemopoiesis in general. The most probable

Table IV *Percentage of scorable metaphases and erythroid mitoses in bone marrow preparations*

Pat. no.	Scorable cells ( )	Erythroid mitoses ( )
I	32	16
II	44	18
III	52	52
IV	21	62
V	42	34

Table V *Comparison between the proportion of erythroid mitoses in the direct marrow smears and the maximal possibility of normal metaphases (explained in text) in the chromosome preparations*

Patients I–V are the cases of the present study patients 1 5 cases reported in previous paper (8)

Pat. no	Maximal possibility of normal metaphases ( )	Erythroid mitoses ( )
I	69	16
II	62	18
III	48	52
IV	84	62
V	62	34
1	41	84
2	65	16
3	61	42
4	40	72
5	41	58

residence of the leukaemic defect is in the common haemopoietic stem cell, as is the case in chronic myelogenous leukaemia (2, 10, 13 14). This would also fit with the high risk of developing AML ("blastic crisis") in chronic myelogenous leukaemia and with the frequent transition of erythroleukaemia to typical AML (3)

Another interesting facet of these data is that, in terms of differentiation block, leukaemic cells in AML are not all alike Some cells remain at an undifferentiated blast level, whereas others go on to differentiate into erythroid cells. Although the data support the above interpretation, it must not be overlooked that the evidence is circumstantial. Final proof will require cytological identification of abnormal karyotypes. By combining karyotype studies with radiouron labelling of cells (9), this should be possible.

Another point which deserves to be studied is whether the chromosome abnormalities in acute lymphoblastic leukaemia are restricted to the leukaemic lymphoblasts, or whether also in this dis ease abnormal karyotypes can be found in dif ferentiating haemopoietic precursor cells. So far we have only had suitable cases of AML and its variants for study

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Acta med. scand. Vol. 189 pp 101–104 1971

QUANTITATIVE ASPECTS OF HEMOLYSIS IN AORTIC VALVULAR DISEASE AND BALL VALVE PROSTHESIS

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Abstract. The degree of hemolysis was evaluated by determination of serum lactic dehydrogenase activity (LDH) in 97 non-selected cases with unoperated aortic valvular disease and 56 patients with Starr-Edwards aortic ball valves. Significant hemolysis was uncommon in unoperated patients. Increased red blood cell destruction was found in the majority of patients with prosthetic valves and exceeded twice the normal in about 35% of the cases. When present, anemia was usually due to iron deficiency. The probable mechanisms of anemia in patients with prosthetic heart valves are discussed.

It is well established that the frequency of hemolysis following insertion of prosthetic heart valves is high (6), but only a few authors have evaluated the degree of the red blood cell destruction (1, 11, 13). It has been shown that determination of serum lactic dehydrogenase activity (LDH) is a suitable tool for evaluation of the degree of intravascular hemolysis in large series of patients with heart valve prostheses (4, 5, 11). We have used this method to study the degree of erythrocyte destruction in a large, non-selected series of patients with aortic valvular disease and aortic ball valve prostheses.

MATERIAL AND METHODS

One hundred and fifty-three non-selected, consecutive patients with aortic valvular disease and Starr-Edwards aortic ball valve prostheses admitted to our Department during 1969 were examined. Patients with additional mitral insufficiency or severe mitral stenosis were excluded, likewise patients with multiple prosthetic valves. Patients with LDH increment from other sources than erythrocytes were excluded as far as possible. Therefore, patients with acute myocardial and pulmonary infarction and cases with severely impaired liver function or elevation of other serum enzymes such as transaminases and phosphatases were excluded from the study.

The series consisted of 32 patients with aortic stenosis, 49 with aortic regurgitation and 16 with combined stenosis and insufficiency. The diagnoses were based on hemodynamic findings. Cases with a pressure gradient across the aortic valve exceeding 25 mm of mercury were classified as aortic stenosis even when a slight aortic regurgitation was seen by angiocardiography. Patients with a significant aortic regurgitation and pressure gradient below 25 mm of mercury were classified as aortic insufficiency.

Fifty-six patients had Starr-Edwards aortic ball valves, in three cases paravalvular leakage was diagnosed. The observation time since operation varied from one month to several years, most patients were examined repeatedly at regular intervals after the operation.

Hemoglobin concentration, red blood cell and reticulocyte counts, serum haptoglobin, bilirubin, iron and the total iron binding capacity of serum were determined by routine methods. LDH was measured according to Wroblewski and La Due (12), the upper normal limit in our laboratory being 200 U/l (μmol/min/l). The red blood cell destruction was predicted from the LDH levels as described elsewhere (5). LDH less than 200 U/l indicated a normal red blood cell survival, whereas values above 500 U/l suggested a destruction rate of twice the normal or more. According to our previous findings (5) an LDH level of 500 U/l corresponds to a half-life of ^{51}Cr-labelled erythrocytes of 18 ± 5 days, that is a real red blood cell life-span of $50 \pm 20\%$ of the normal (mean ± S.D.).

RESULTS

Fig. 1 shows the main hematological findings in the different groups of patients. The hemoglobin concentration was less than 12 g/100 ml in only four unoperated patients, in three cases anemia was caused by iron deficiency and in one by active rheumatoid spondylitis. Reticulocyte counts of 2% or more were recorded in seven cases, haptoglobin concentration was below 15 mg/100 ml in 13 and serum LDH exceeded 200 U/l in 26 patients. However, the LDH increment was slight in most cases, the values being just above

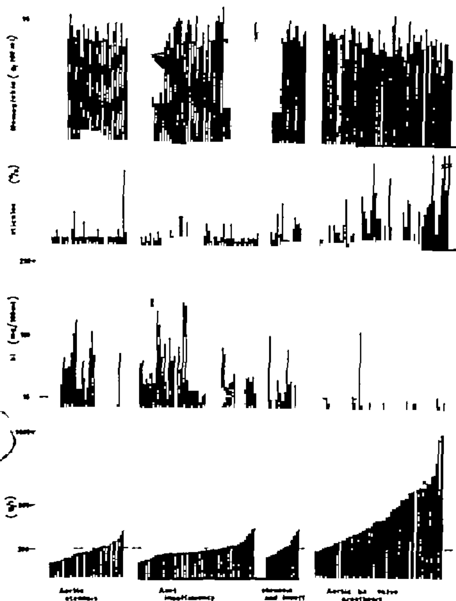


Fig. 1. Hematological data of the material. The individual cases are arranged according to increasing LDH values in each group. Patients with incompetent prostheses are indicated ( ).

the upper noral limit. LDH, haptoglobin and reticulocyte values did not differ significantly between the groups of aortic stenosis, aortic regurgitation and combined stenosis and insufficiency. Hemolysis was not significantly related to the severity of the valvular lesion in the unoperated patients (Fig. 2).

Increased LDH values and haptoglobin depletion were found in 52 of the 56 patients with aortic ball valves, suggesting the frequency of hemolysis to be 93%. In 19 cases LDH exceeded 500 U/l, indicating a red blood cell destruction of twice the normal or more in about 30% of the operated patients. In four cases the LDH increment suggested a hemolytic activity of three to four times the normal. The reticulocyte counts exceeded 2% in only 26 cases.

The serum haptoglobin fell to levels near zero in very low-grade intravascular hemolysis and give no information about the severity of red blood cell destruction. The reticulocyte counts showed the same trend as LDH, but the individual values varied considerably. Hemoglobin concentration decreased moderately with increasing hemolysis as predicted from the LDH values. Only in nine cases, however, were values below 1 g/100 ml observed. Megaloblastic anemia or folic acid deficiency was not seen in this series.

Hemolysis was not higher in three patients with paravalvular leakage than in patients with competent ball valves. A moderate hemolysis remained unchanged in one patient who was reoperated on because of valve leakage. Two patients in this series developed severe hemolytic anemia about three months after the operation there were no signs of paravalvular leakage or ball variance in these cases. Neither infection nor immunohemolytic mechanisms could be demonstrated The hemoglobin concentration decreased rapidly LDH rose to 2 420 and 1 870 U/l and the half-life of their own [51]Cr-labelled erythrocytes shortened to six and nine days, respectively Hemolysis decreased and hemoglobin values rose during prolonged bed rest and iron treatment in both cases.

Fig. 3 shows that patients with hemoglobin values below 12 g/100 ml were all iron-deficient, except one who had recently recovered from a bacterial endocarditis. The relation between iron deficiency and degree of hemolysis is not quite clear Of the 14 patients with iron deficiency anemia, only five had LDH levels indicating a hemolysis of more than twice the normal.

DISCUSSION

Normally the bone marrow may compensate for a red blood cell destruction of three to four times the normal if sufficient iron is available (3) However even an erythrocyte turnover exceeding twice the normal imposes constant stress on the bone marrow and increases the demands for iron and

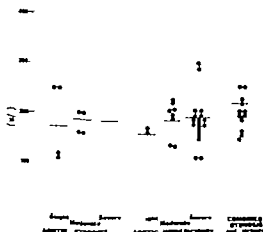


Fig 2. LDH values related to the severity of the aortic valvular lesion in unoperated patients.

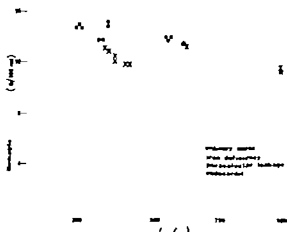


Fig 3 The relationship between hemolysis as predicted from the LDH levels and hemoglobin concentration in patients with prosthetic aortic valves.

folic acid. In the present series a red blood cell destruction more than twice the normal was seen in about 15% of the patients with aortic ball valves, a figure in agreement with others (2, 11) using different techniques. Clinically important hemolysis was not observed in unoperated cases.

Several authors consider prosthesis incompetence to be the cause of severe hemolysis in the majority of cases (1 8, 11). Our data do not confirm this opinion. In three patients with significant paravalvular leakage the hemolysis was similar to that in patients with competent valves, whereas no faults of the prosthesis were detectable in our two patients who developed severe hemolytic anemia. The incidence of ball valve incompetence is surprisingly high in several reports (1 11 13): this might be due to a selection of patients with malfunction of the prostheses. In our hospital paravalvular leakage was seen in only 6 of 57 patients with Starr Edwards aortic ball valves inserted during 1965–68 reoperation with closure of the leakage was performed in three of the cases (10). The majority of patients operated on are later examined at regular intervals in our department. Our rather low frequency of paravalvular leakage might be due to routinely performed flowmetry immediately after the valve insertion and primary correction in the event of regurgitation (9).

The tendency to develop anemia increased significantly with increasing hemolysis. In the ma

jority of cases the anemia was of the iron-deficiency type, in spite of supplementary iron given to most patients since insertion of the prosthesis. Therefore, anemia in patients with Starr Edwards aortic ball valves should not be regarded as a pure hemolytic anemia, but as a combined hemolytic and iron-deficiency anemia.

In our opinion, the initiating factor is intravascular hemolysis caused by mechanical trauma of the ball valve, crushing of the erythrocytes between the ball and the valve site probably being more important than turbulence of the blood stream. Intravascular hemolysis leads to extra ordinarily high urinary excretion of iron (11) and development of iron deficiency The bone marrow in accelerated erythropoiesis is very sensitive to iron deficiency even moderate iron deficiency may depress erythrocyte production (3). Probably iron-deficient microcytes are more fragile than normal red blood cells. Thus a vicious circle is formed, increased mechanical hemolysis results in extraurinary iron loss, iron deficiency causes increased red blood cell fragility and depresses the formation of new erythrocytes. This hypothesis may explain the development of hemolytic crises and the good response to iron treatment in some of these patients (7 8)

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MECHANISMS OF HEMOLYSIS IN PATIENTS WITH HEART VALVE PROSTHESES

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Abstract. Intravascular hemolysis was studied in large, non-selected series of patients with aortic and mitral valvular disease or valve prostheses in order to clarify the mechanisms of the erythrocyte destruction. Hemolysis was slight in unoperated patients, even in cases with severe valvular disease. The degree of erythrocyte destruction in operated patients was mainly determined by the design and material of the prostheses, whereas it was similar in cases with aortic and mitral valves replaced. Our data suggest that the hemolysis in patients with prosthetic heart valves mainly is due to crushing of erythrocytes by the mechanical components of the prostheses, turbulent blood flow probably being rather unimportant factor.

The mechanisms of the hemolysis following prosthetic heart valve replacement are not clear. The general view is that turbulence of the blood flow through the prosthetic valves is a main determinant of red blood cell destruction (1 9 11 13 15).

In order to clarify these mechanisms we have reconsidered our data about the degree of hemolysis in a large, non-selected series of patients with valvular heart disease and prosthetic valves. Some of these data have been published previously in the discussion of other aspects of hemolysis in patients with valve prostheses (5 6, 7).

The hemolysis was evaluated by determination of the serum lactic dehydrogenase activity (LDH), a method previously found convenient and reliable (4).

MATERIAL AND METHODS

All patients ith aortic and mitral valvular disease or valve prostheses seen during 1969 were examined. Patients with combined aortic and mitral valvular disease, and patients with LDH increment from other potential sources than erythrocytes, were excluded. Two hundred and thirty-four cases ere studied, 97 ith aortic and 48 with mitral valvular disease, 64 with aortic and 21 with mitral valve prostheses, and 4 cases with both aortic and mitral valves replaced.

The LDH was determined by conventional method (14), and the degree of intravascular hemolysis as predicted as described elsewhere (4). LDH levels below 200 U/l indicated normal red blood cell survival, hereas values exceeding 500 U/l suggested hemolysis of twice the normal or abov

RESULTS

The LDH levels in the different patient groups are shown in Table I. Hemolysis was absent or slight in unoperated valvular heart disease. LDH exceeded 500 U/l in only two cases, both suffering from mitral regurgitation.

Hemolysis was increased to twice the normal or above in 30–40% of patients with prosthetic heart valves: there was no difference between patients with aortic and mitral valve prostheses. Within either group the individual values differed considerably chiefly depending on the type of valve inserted. In patients with aortic ball valves the Starr-Edwards 2300 model provoked twice as much hemolysis as valves of the older 1200 series and Magovern valves. The Beall mitral valves provoked more than twice as much erythrocyte destruction as the Starr Edwards mitral valves. The LDH levels were similar in aortic and mitral valves of the Starr Edwards type with silastic ball pellets. Thus the type of valv prosthesis inserted influenced hemolysis much more than its localization. In the few patients with both aortic and mitral valves replaced, hemolysis was increased to the double of that seen in cases with single valve prosthesis.

In order to study the influence of the valvular lesion on hemolysis, cases with slight and severe

Table I. *The LDH lev ls (U/l) in aortic and mitral valvular disease and valve prostheses*

	No. of cases	Mean LDH	No. of cases with LDH < 200	200–500	> 500
Valvular heart disease					
Aortic					
Stenosis	32	185	21	11	0
Regurgitation	49	183	37	12	0
Stenosis + regurg.	16	218	8	8	0
Total	97	192	66	31	0
Mitral					
Stenosis	12	187	9	3	0
Regurgitation	10	255	6	2	2
Stenosis + regurg.	26	193	17	9	0
Total	48	207	32	14	2
Valve prostheses					
Aortic					
Magovern	8	245	0	8	0
Starr Edwards 1200	13	281	2	10	1
Starr Ed ards 2300	43	501	0	25	18
Total	64	424	2	43	19
Mitral					
Magovern	1	275	0	1	0
Starr Edwards	7	277	1	6	0
Beall	13	625	0	4	9
Total	21	492	1	11	9
Multiple	4	909	0	0	4

aortic stenosis and aortic regurgitation, respec tively were selected. Table II shows that hemolysis was sl ghtly influenced by the severity of the valvular disease. Furthermore, in our few cases with paravalvular leakage the hemolysis was only slightly higher than in competent valves. Leakage seemed to influence hemolysis somewhat more in cases with mitral than aortic valves.

DISCUSSION

Our results strongly suggest that intravascular he molysis is caused by crushing of the erythrocytes in the prosthetic valves rather than by turbulence of the blood forced through th prostheses. Firstly even greatly diseased valves provoked slight hemolysis as compared with properly functioning valve prostheses. In severe aortic stenosis angiocardiography showed a great turbulence of the blood flow however hemolysis in such cases was not significantly higher than in slight valvular stenosis without turbulent blood flow Also in aortic re gurgitation the hemolysis was only slightly influenced by the degree of turbulence. If turbulence was a main determinant of hemolysis, a much higher degree of hemolysis might be expected in unoperated valvular disease.

Secondly in aortic ball valves the material of the prosthesis greatly influenced hemolysis. Re placement of the silastic rubber pellet by a me tallic ball and teflon-coating of the ring and cage in the Starr Edwards valves, increased the hemolytic effect seriously (5). The most important dif ference between the two types of Starr Edwards valves *probably is the material of the pellet* hemolysis was similar in Magovern and Starr Edwards valves with silastic rubber balls. The turbulence caused by the two types of Starr Edwards valves most probably is similar therefore hemolysis is best explained by a direct traumatic ef fect on the erythrocytes. The different hemolysis provoked by Beall and Starr Edwards mitral valves suggests that also the shape of the pellet is important, but the relative importance of turbulence and crushing effect in these two valves is not so clear

Thirdly the similar degree of hemolysis follow ing aortic and mitral valve replacement fits best with a crushing effect caused by the mechanical components of th prostheses. Turbulence is marked when blood is forced through an aortic ball valve in systole (12), whereas it must be negligible when the blood passes through the

Table II. *The LDH levels (U/l) in selected cases with slight and severe aortic valvular disease and in patients with valve leakage and competent valve prostheses*

	No of cases	Mean LDH
Aortic stenosis (pressure gradients)		
< 25 mm Hg	6	145
> 50 mm Hg	7	170
Aortic regurgitation (angiocardiographically)		
Slight	4	164
Severe	25	184
Prosthetic aortic valves		
Competent	61	408
Leakage	3	481
Prosthetic mitral alves		
Competent	18	465
Leakage	3	587

mitral valve during diastole. However closure of the prosthetic valve might be expected to damage the erythrocytes similarly in aortic and mitral valves. The force by which the pellet is flung back against the valve ring at valve closure is of the same order of magnitude in aortic and mitral valves. Furthermore, as aortic and mitral valves provoked similar erythrocyte damage the twofold increase of hemolysis in patients with two prosthetic valves was to be expected.

Although the direct traumatic effect on the erythrocytes in our opinion is the main determinant of hemolysis, turbulent blood flow might be an important additional factor in some cases with prosthetic heart valves. Other factors such as ball variance (2, 3), physical activity (8) and probably iron deficiency (10) may also influence the hemolysis in these patients.

ACKNOWLEDGEMENT

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THE EFFECT OF GLUCOSE ON THE GLOMERULAR FILTRATION RATE IN NORMAL MAN

A Preliminary Report

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Abstract. On ten occasions the renal clearances of inulin and ^{51}Cr-EDTA have been measured before, during and after hyperglycemia induced in nine normal young men. Glucose was given as combined oral and intravenous load, so that the blood glucose concentration was elevated moderately for 2–3 hours. During the hyperglycemia there was a highly significant increase, averaging about 9%, in the inulin and ^{51}Cr-EDTA clearances. The maximum increase in inulin clearance averaged 18% and in ^{51}Cr-EDTA clearance, 19%. The plasma volume increased, on average, 16%. The effect of glucose loading on the glomerular filtration rate may be explained either by haemodynamic factors or by an interaction between glucose and sodium transport in the kidney.

It has recently been shown that the glomerular filtration rate (GFR) in young diabetics without nephropathy is higher than normal (3–5). To examine the significance of glucose for the high GFR, the renal clearances of inulin and ^{51}Cr-EDTA were measured in normal subjects under glucose load. GFR was found to increase during hyperglycemia.

MATERIAL AND METHODS

On ten occasions the sodium excretion and the renal clearances of inulin and ^{51}Cr-EDTA were measured before, during and after hyperglycemia induced in nine normal men aged from 22 to 30 years. The examination lasted from 9 a.m. to 3 p.m. The subjects were lying in supine position and had fasted overnight. The bladder was emptied by spontaneous voiding. The sodium concentration was measured with a flamephotometer (Eppendorf), and inulin by Bojesen's method (1). Before the analysis, inulin and glucose in serum were separated by gel filtration (G-25 sephadex in H_2O). In urine samples with glucose, the inulin concentrations were corrected by the inulin equivalent for glucose (1.22%). The activity of ^{51}Cr in serum and urine was determined in a well-type scintillation counter. At least 10 000 counts were recorded.

Changes in the plasma volume were calculated by measuring, at the end of each clearance period, the colloid osmotic pressure in serum with a Tybjerg Hansen osmometer (6). During the whole examination period the glucose concentration in capillary blood was measured with glucose oxidase in an Auto-analyser.

After three control periods, 1 g of glucose per kg body weight was given orally. In seven cases an additional intravenous infusion of 5.4–10 ml of 5.5% glucose/min was given 45–60 min later during 90 min. There were four to six 30-min clearance periods during hyperglycemia, and one to three periods after hyperglycemia. The duration of hyperglycemia is here defined as the time from the oral glucose administration to the end of the last clearance period in the middle of which the blood sugar concentration exceeded the fasting value. At the beginning of the examination the subjects were given 1 l of water to drink, and during the examination a similar amount every hour. During the intravenous glucose administration the subjects were not given anything to drink.

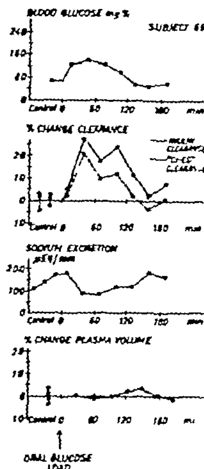
RESULTS

Representative data from two subjects are shown in Fig. 1. It is seen that the inulin and ^{51}Cr-EDTA clearances varied with the blood sugar concentration. Sodium excretion rose during the control periods, falling again during the first periods after the oral glucose administration. The plasma volume increased during the infusion of glucose, decreasing towards the control value after the end of the infusion. In the case where glucose was only given orally, the plasma volume did not change.

As a whole the hyperglycemia lasted 2 to 3 hours. The blood sugar concentration reached its peak value of 140–250 mg/100 ml in the middle

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1



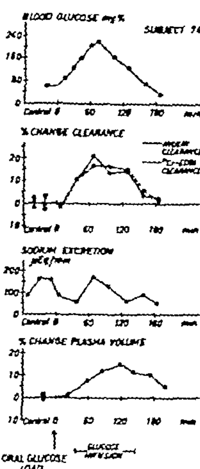


Fig. 1. The effects of oral (*left*) and combined oral and intra enous glucose load (*right*) on the renal clearances of inulin and [51]Cr-EDTA, urinary sodium xcretion, and plasma volume in t normal subjects.

of the period. In seven cases glycosuria occurred in three or four clearance periods, the maximal excretion of glucose was 10–60 mg min.

Table I shows that during the hyperglycemia there was a highly significant increase averaging about 9% in the inulin and Cr EDTA clearances, while the clearances did not differ significantly before and after the hyperglycemia. The maximum increase in inulin clearance averaged 18% (5–27), and in [51]Cr EDTA clearance 19% (10–24).

From the first to the third control period, sodium excretion *increased* on an average *from* 123 to 192 µEq min. From the first to the second clearance period during hyperglycemia, excretion fell from 205 to 112 µEq/min, and *during* the last period with hyperglycemia, averaged 141 µEq/min.

A significant change in the plasma volume during the hyperglycemia occurred only in the seven cases in which combined oral and intravenous administration of glucose was given. 120–150 min after the oral administration the plasma volume had increased, on average by 16% (12–26).

DISCUSSION

The increase in inulin clearance was of the same order of magnitude as that reported by Brod et al. (2) and by Ek (4) following intravenous infusion of approximately 1 g min of glucose. As simultaneous changes in Cr EDTA clearance were observed *in the present work*, it can be concluded that GFR increases during hyperglycemia. *The increase may be due to an expansion of the* extracellular volume (ECV), as proposed by Ek (4) and by Brod et al. (2) or may be explained by an interaction *between the glucose and sodium* transport in the kidney (7, 8).

Table I. *Inulin and* ^{51}*Cr-EDTA clearances before (C), during (H), and after (AH) hyperglycemia*

	Inulin clearance (ml/min)			^{51}Cr-EDTA clearance (ml/min)		
	C	H	AH	C	H	AH
Mean	131.5	143.4	130.9	116.0	127.1	114.3
Mean diff. from control	—	11.9	0.6	—	11.1	1.7
S.D. mean diff.	—	7.0	11.2	—	3.7	7.7
No. of subjects	9	9	9	10	10	10
Significance of difference from control, p	—	<0.001	>0.1	—	<0.001	0.1

Sodium excretion increases in man during acute intravenous infusion of saline. In the present work, sodium excretion was found to decrease, whilst in the examinations by Ek it was unchanged. This shows that the response by the kidneys to an ECV expansion caused by glucose differs from that caused by saline. However this does not preclude that the increase in GFR following infusion of glucose is released by the ECV expansion. The possibility that there is another explanation of the phenomenon is suggested by the finding that, in the three subjects who were only given glucose orally the inulin and ^{51}Cr EDTA clearances rose without simultaneous plasma volume expansion.

In isolated frog kidneys, Vogel and Kröger (7) showed that glucose reabsorption is dependent on sodium reabsorption, a high sodium reabsorption rate being followed by a high glucose reabsorption rate, whereas sodium transport is less dependent on glucose transport (8).

If there is a constant relationship between sodium reabsorption and GFR, the findings by Vogel et al. (8) might support the view that the increase in GFR during hyperglycemia is due to glucose acting on the sodium reabsorption, which in turn gives rise to an increase in GFR.

The 18% increase in GFR during moderate hyperglycemia demonstrated in the present paper is of the same order of magnitude as that found in young diabetics without nephropathy (3, 5). It is reasonable to suppose a joint explanation for the relationship between blood sugar concentration and GFR demonstrated under the two conditions. Based on the present material, it is impossible to decide whether the changes in GFR are determined by hemodynamic factors or released via the sodium transporting cells.

ACKNOWLEDGEMENTS

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THE VARIATION IN TWENTY-ONE SERUM PROTEINS BEFORE AND AFTER RENAL TRANSPLANTATION

I *General Pattern*

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Abstract. The concentration of 21 serum proteins was followed by immunochemical methods prior to and after kidney transplantation in 21 consecutive patients. In all 21 patients several proteins deviated from normal before as well as after the transplantation. The material includes one patient who received a kidney from an identical twin, and five patients who had an uneventful postoperative course. Analysis of the data shows that the changes in serum protein levels following renal transplantation are not caused by 1) the homograft reaction, 2) the immunosuppressive treatment; 3) complications in the postoperative course. The metabolism of several serum proteins seems to be changed when a previously uremic patient receives a kidney transplant. Further studies with labelled proteins are necessary to evaluate the problem in greater detail.

Studies on the serum protein pattern in relation to renal transplantation have been exclusively concerned with the determinations of only a few proteins (e.g. complement, the immunoglobulins) in relation to rejection episodes (1). Many factors may however cause changes in the serum proteins before as well as after grafting, e.g. 1) the complete abolition of the uremic state, 2) the change from a restricted to a full diet; 3) the immunosuppressive treatment and 4) complications other than rejection episodes. In order to elucidate whether specific changes occur in relation to rejection, it was deemed necessary to examine many proteins before and by repeated examinations after renal transplantation.

This paper reports on the variation in 21 serum proteins in 21 consecutively transplanted patients in order to illustrate a general pattern from which changes occurring during rejection episodes can be evaluated. The changes occurring during rejection are dealt with in a following paper (11).

METHODS

The immunoelectrophoretic methods have been described previously (2, 5, 7, 8, 9) and will therefore be referred to only very briefly.

Electrophoresis in antibody containing agarose a.m. Laurell (5, 7)

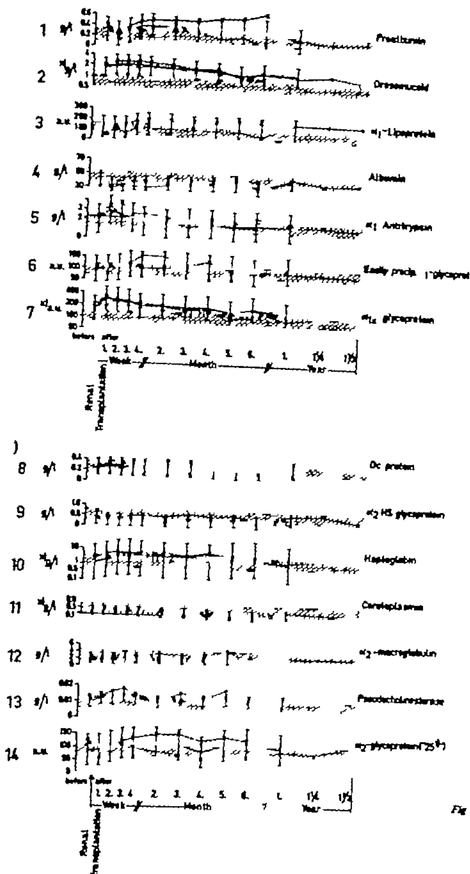
Based on double analyses, serum albumin, IgG, IgA and IgM are determined by electrophoresis in agarose containing rabbit antihuman albumin, IgG, IgM (Dakopatts A/S) and IgA (Behringwerke). The mobility of the immunoglobulins during the electrophoresis is altered after carbamylation (7). The coefficient of variation on repeated determinations of a normal serum during a long period range from 3 to 6%.

The Laurell crossed antigen-antibody electrophoresis a.m. Clarke and Freeman (2, 7, 8, 9)

A mixture of equal volumes of serum and reference (carbamylated transferrin (8)) is separated by electrophoresis in agarose followed by electrophoresis at right angles in a gel containing rabbit antihuman serum proteins (Dakopatts). Of the approximately 50 proteins which are identifiable as precipitate arcs, 17 have been determined in this study. The area under an individual precipitate, which is directly proportional to the protein concentration (2), is integrated on a semiautomatic planimeter and compared with the area under the corresponding precipitate in the serum of reference. The coefficient of variation on repeated determinations of all proteins was 10% (range 3–21%).

Reference. A pooled serum from 1 000 normal blood donors was stored at −18°C with sodium azide (1‰ w/v) and used as reference, the concentration being fixed at 100 arbitrary units (au). On this basis the concentrations of the unknown sera could be expressed in au. With reference to "Standard Serum op. nr. 166 (Behringwerke)" the concentrations of 16 serum proteins were converted to g/l. The remaining 5 proteins are stated in au.

Patient sera were stored at −18°C after addition of 1‰ sodium azide until immunochemical analysis was carried out.



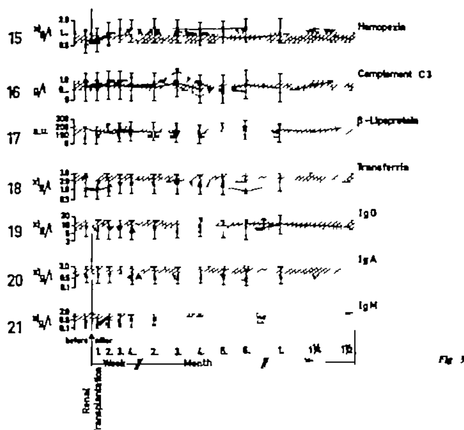


Fig. 3

MATERIAL

Data on 21 consecutively kidney-transplanted patients have been presented in a previous report (12), and the patients are referred to by the same numbers (nos. 1–21). The treatment was carried out between Jan. 1968 and Sept. 1969 at Rigshospitalet. The time of observation after the transplantation was from one to 18 months. The average age for nine women was 31 years (17–45 years), for 12 men 39 years (21–59 years).

Nineteen patients were bilaterally nephrectomized before transplantation. All patients were in chronic hemodialysis twice weekly. As immunosuppressive treatment before the transplantation, 18 patients received extracorporeal irradiation of the blood (13).

After the transplantation the 20 allotransplanted patients received immunosuppressive treatment with prednisone (30–20 mg/day during the first months, the dose being later reduced to less than 10 mg/day) and azathioprine (Imurel® 75–150 mg/day). Seventeen patients, in addition, received brief treatment with extracorporeal irradiation of the blood. Patient no. 1, who received a kidney from an identical twin, had no immunosuppressive treatment.

In five patients (nos. 6, 10, 11, 18 and 20) the clinical course after the transplantation was uneventful.

In the remaining 16 patients one or more of the following postoperative complications occurred: *Episodes of rejection* (nos. 2, 3, 5, 7, 14 and 17) during which the dose of prednisone was temporarily increased to 100–150 mg/day; *ureteral leakage with urosepsis* leading to reoperations (nos. 1, 3, 13 and 21); *bacterial infections* (nos. 3, 4, 7, 9, 15 and 16); *acute fatal serum hepatitis* (no. 8); *migrating thrombophlebitis* (no. 12); and *arterial hypertension* due to arteriosclerosis of the renal artery (no. 19).

As several serum proteins vary according to sex and age, sera from 21 normal persons of the same sex and age as the patients were used as controls.

Before transplantation, sera—taken prior to hemodialysis—were examined in 20 patients. After transplantation a total of 140 sera were examined from the 21 patients.

Calculations

The statistical analysis was performed on a Fortran programme BMD 07D (Biomedical Computer Program, May 1966) at NEUCC, Lundtofte, Denmark. In none of the 21 serum proteins examined the arithmetic values in controls and in patients showed a positive skew distribution, whereas the logarithmically transformed values were closer to a Gaussian distribution. In these cases, therefore, the logarithmically transformed values were used for the calculations. The results obtained for the proteins in the patients before and at regular intervals after renal transplantation were pooled and compared with the con-

trol group by means of the Student t-test. For each protein the S D of the control group was compared with an average S.D for the patients by means of the F-ratio test. On the basis of the quantitative protein determinations in the 160 patients sera and the 21 control sera the coefficient of correlation (r) between the individual proteins in pairs was calculated in the two groups.

RESULTS

In Figs. 1 2 and 3 the *mean values* ± 2 *S.D* of 21 serum proteins in the patients before and after transplantation are indicated in relation to the corresponding values of the control group. Patient no. 1 who did not receive immunosuppressive treatment, and the five patients with uneventful clinical course are included in the figures.

The proteins are numbered according to the Figs. 1 2 and 3 and will be commented on with reference to these numbers below

Examination of variance With respect to 14 serum proteins (nos. 1 2, 3 5 6 7 10 13 14 15 16 17 18 and 19) there was a larger variance in the patients than in controls. Only with respect to ceruloplasmin was the variance less in the patients.

Before transplantation mean concentrations of proteins nos. 1 2 3 5 and 7 were *increased* whereas those of nos. 4 9 11 12, 15 17 18, 19 20 and 21 were *decreased* as compared to the controls. In three patients (nos. 2, 4 and 21) haptoglobin could not be demonstrated (<0 01 g/l)

First and second week after transplantation. proteins nos. 1 4 9 18, 19 and 20 were *decreased* whereas nos. 2, 5 6 7 and 15 were *increased*

After the second week following transplantation proteins nos. 1 2, 3 and 7 were *constantly elevated* nos. 6, 10 13 14 and 15 *temporarily elevated* In the same period the serum concentrations were *constantly below* normal for proteins nos. 9 11 18 and 20 and *temporarily reduced* for nos. 4 12, 19 and 21

From the patient group some well defined clinical courses are extracted.

Course in patient no 1 Th. uneventful periods were associated with *elevated* concentrations of proteins nos. 1 2, 5 6, 7 10 and 13 and *reduction* of nos. 4 18 and 20 The elevation of protein no 1 was of shorter duration and for no. 7 less pronounced than in the other patients. The concentrations of proteins nos. 9 11 12, 14 and 15 did not differ from the control group. In connection with *reoperations* in the first and second week after transplantation, protein alterations of the same type as following transplantation were observed. *A febrile metrorrhagia* six months after the transplantation was associated with falling concentrations of proteins nos. 1 3 4 9 12 and 18 and rising concentrations of nos. 2, 5 7 and 10

Uncomplicated clinical course In the five patients the elevation of proteins nos. 1 2, 3 10 14 and 15 was more pronounced than in the other patients. The elevation of protein no 7 was less pronounced and the concentrations of nos. 4 18 and 19 more quickly normalized.

Minor complications (reoperations, slight and transient fever) with good general condition were followed by reduced concentrations of protein nos. 1 4 and 18 and increased concentrations of nos. 2, 5 7 10 and in some cases no. 8.

Major complications (severe infections, uroplania) with impaired general condition showed similar alterations, although more pronounced. Moreover elevated concentration of protein nos 6 14 15 16, 19 and 21 and low concentrations of nos. 8 9 12 and 17 were frequently found. One case of *hepatitis* was associated with falling concentrations of all serum proteins except the immunoglobulins, which increased.

Examination of correlation. The presence of significant ($p<0.01$) positive correlation between the concentrations of two proteins tells us that low and high values for one protein respectively are associated with low and high values for the other protein. In the patient and control groups a significant positive correlation was found b. tween proteins nos. 2/7 5/7 and 19/20 In the control group there was a positive correlation between proteins nos. 2/16, 6/8 7/10 and 13/16 while in the patient group positive correlation between nos. 1/3 1/14 1/15 1/18 2/5 3/15 3/18 6/12, 6/16, 8/12, 8/16, 12/13 14/16 15/18 and negative correlation between nos. 4/7 was observed.

DISCUSSION

The study shows that the concentrations of several serum proteins are changed prior to and after renal transplantation.

Prior to the transplantation there may be many

reasons for the altered protein pattern. The patients were uremic (19 of 21 nephrectomized), treated with diet (poor in protein and calcium, rich in lipid), anticoagulation (Marcoumar®), intermittent hemodialysis and extracorporeal irradiation of the blood (18 of 21 patients prior to the transplantation).

The uremic condition per se has an immunosuppressive effect and might explain the reduced concentration of the three immunoglubulins (3 15). The extracorporeal irradiation damages primarily the circulating lymphocytes and not the immunoglobulin synthesis nor the other proteins (10) The reduced concentration of albumin and transferrin could be a consequence of the protein-restricted diet, and the low calcium diet could be associated with the low concentration of the calcium-binding alpha-2 HS glycoprotein, as it has been found that the addition of calcium to the diet increases the concentration of this protein in serum (10). The reduced concentrations of haptoglobin and hemopexin in several patients may very well be ascribed to increased intravascular hemolysis in dialysed uremic patients (4). Increased cell destruction during the hemodialytic treatment and inflammatory reactions around the shunts cannot be disregarded as a possible cause of the slightly elevated concentrations of some acute phase proteins (orosomucoid, alpha-1 antitrypsin, alpha-1 -glycoprotein). The increased concentration of the thyroxin-binding prealbumin may reflect an alteration of the hormonal balance in the uremic organism.

After the transplantation several factors may explain the alterations in the serum proteins, viz. the surgical intervention, the abolition of uremia and the change to sufficient diet, the homograft reaction, the immunosuppressive treatment and postoperative complications.

An uncomplicated surgical intervention (e.g. appendectomy) will cause a slight fall in the concentrations of prealbumin, albumin, complement C'3 transferrin and IgA, a slight increase in orosomucoid, alpha-1-antitrypsin, easily precipitable glycoprotein, alpha-1 -glycoprotein, haptoglobin and hemopexin, alterations which have occurred after two weeks (10 14). The alterations after transplantation are more pronounced and comprise more serum proteins (alpha-2 HS glycoprotein, pseudocholinesterase, IgG).

The homograft reaction could be supposed to cause part of the prolonged alterations. For evaluation of this problem the course in patient no. 1 who received a kidney graft from an identical twin is of special interest. The results indicate, however that the changes were similar to those seen in the allografted patients. Similarly the immunosuppressive treatment did not cause any particular alterations. Immunosuppression, however, may explain the difference between patient no. 1 and the five patients with uneventful postoperative course (in the latter elevated prealbumin, orosomucoid, alpha 1-lipoprotein, alpha-1 -glycoprotein, alpha- -glycoprotein and hemopexin, lower alpha-1-antitrypsin and alpha-2 HS glycoprotein).

In connection with minor complications which involved cell destruction of limited extent there was increased concentration of some acute phase proteins (orosomucoid, alpha-1-antitrypsin, alpha-1 -glycoprotein), and slightly reduced concentration of prealbumin, albumin and transferrin. In severe complications in which the cell destruction was more comprehensive these alterations were more pronounced and covered a larger number of serum proteins (increased—easily precipitable glycoprotein, haptoglobin, alpha-₂-glycoprotein, hemopexin, complement C'3 IgG and IgM reduced—alpha-1-lipoprotein, alpha- -HS glycoprotein, Gc protein, alpha-2-macroglobulin and beta-lipoprotein). In one case of hepatitis reduction of all serum proteins except immunoglobulins was observed. This strongly suggests that many of these proteins are synthetized in the liver The fact that the concentration of some serum proteins was increased during some complications and reduced during others (Gc protein, hemopexin, alpha-2-glycoprotein and pseudocholinesterase) might be due, inter alia, to more or less pronounced impairment of the protein synthesis in the liver

The fact that significant correlation was found between the concentrations of several serum proteins suggests a functional and metabolic interdependence. The so-called acute-phase proteins which are characterized by increasing concentration after variety of influence, seem to comprise easily precipitable glycoprotein and alpha-1 -glycoprotein. The other group of proteins which, during similar influence, shows falling concentration comprises prealbumin, alpha-1-lipoprotein, albumin alpha-2 HS glycoprotein, hemopexin, beta-lipoprotein and transferrin. Gc pro-

tein, alpha-2-glycoprotein and complement C'3 occupy an intermediary position, their concentrations being positively correlated to the proteins within each of the said groups. These three proteins are most frequently elevated during slight complications and become reduced later in the clinical course or by pronounced influences. Al though IgA is permanently reduced during the postoperative period in the transplanted patients, there is still a positive correlation between the concentrations of IgA and IgG as in normal subjects, which suggests that the alteration of the transplanted previously uremic organism is of a quantitative and not a qualitative character

It is difficult to base a conclusion concerning the metabolism of a protein on the serum concentration, as the latter is the resultant of synthesis, catabolism and distribution between the extra- and intravascular spaces. Although there are strong implications that the metabolism of several serum proteins is changed, when previously uremic patients receive a kidney transplantation, an evaluation of this question can be made only on the basis of metabolic studies with labelled proteins.

ACKNOWLEDGEMENTS

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THE VARIATION IN TWENTY-ONE SERUM PROTEINS BEFORE AND AFTER RENAL TRANSPLANTATION

II. *Changes during Acute Rejection*

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Abstract. The concentrations of 21 serum proteins were followed by immunochemical methods during ten acute rejection episodes after renal transplantation. A significant fall in the concentrations of albumin and Gc protein was demonstrated during the rejection episodes. However, similar changes may occur in other conditions and therefore cannot be considered specific for rejection. Minor alterations in the remaining 19 of the proteins examined were non-significant. A comparison between the protein changes in early and late rejections, and a study of the changes in one patient who had both an early and late rejection, clearly showed a difference between the changes of the serum protein pattern during early and late rejection.

In a previous study the general pattern of serum proteins was shown to be markedly changed after kidney transplantation, and the causative role of various complications and treatment was discussed (7).

In association with rejection episodes following kidney transplantation several workers have reported deposition of certain proteins (gammaglobulin, complement, fibrin) in the renal parenchyma (1–5). Moreover change of low molecular proteins which are catabolized in the renal tubules (lysozome, Bence-Jones protein) has been reported to occur during acute rejection (3, 4). It must be assumed that several other protein changes are associated with rejection of a renal allograft.

The present study is especially concerned with changes of the serum protein pattern during episodes of acute rejection and reports the alterations of 21 serum proteins followed during acute rejection episodes in ten patients who had received a kidney allograft.

METHODS

Serum albumin, IgG, IgA and IgM were determined by electrophoresis in antibody containing agarose according to Laurell (7). The other 17 serum proteins (see Fig. 1) were quantitated by means of the Laurell crossed electrophoresis according to Clarke and Freeman as previously described (7).

MATERIAL

In 10 of 34 kidney-transplanted patients presented in a previous study (8) the serum proteins were followed during acute rejection episodes. This was possible because blood was drawn from all transplanted patients at repeated intervals and serum was stored at −18°C. The diagnosis of rejection was based upon the usual clinical criteria (6) and confirmed by renal biopsy. The data of the patients and the time of rejection after the transplantation are shown in Table I.

Four representative samples during the rejection episodes were examined in all patients: 1) before symptoms of rejection; 2) during progression; 3) during remission; and 4) after normalization of the renal function.

The rejection was treated with prednisone, Imurel, and in four patients (nos. 5, 14, 23, 31) with extracorporeal irradiation of the blood. The doses of prednisone and Imurel on the days of investigation are stated in Table II. In nine of the patients the renal function was normalized after the episode. In one (no. 17) renal function remained reduced and the subsequent course was characteristic of "chronic" rejection.

RESULTS

Six patients developed symptoms of acute rejection during the first week after transplantation (mean, fifth day). Four patients had their rejection episode 1½–6 months after transplantation (mean: third month).

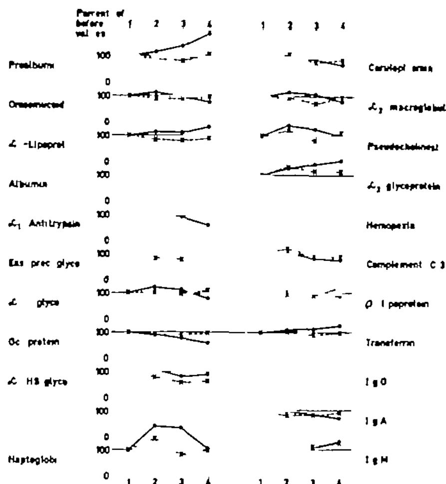


Fig. 1 The variation in 21 serum proteins before 1), during progression 2), during remission 3) and after 4) rejection episodes in six patients with early (●—●) and four patients with late onset (--). The concentrations expressed in % of the values before rejection (see Table III).

In Table III the serum concentrations (mean and S.D) before the rejection episode are compared to the values of the first week and the third month after transplantation in patients with an uncomplicated course (7) The normal range in 21 controls is indicated.

Fig. 1 presents the variations of the 21 serum

Table I. *Data of ten patients with acute rejection episodes*

Pat. no.	Age	Sex	Days of rejection episode
2	45	♀	124–130
3	28	♂	7 25
5	36	♂	47 55
7	51	♂	147 160
14	40	♀	7 17
17	59	♂	64–
22	33	♂	3–5
23	37	♂	4–7
31	44	♀	6–21
34	52	♂	5–12

proteins. The values are expressed in percentage of the first determinations. The changes in protein concentrations were tested by analysis of variance. *During early rejection* in six patients the changes for prealbumin (+87%) and Gc protein (−40%) were significant ($p<0.05$ resp <0.01). The *increasing* concentrations of haptoglobin (+90%) pseudocholinesterase (+41%) alpha-2-glycoprotein (+59%) hemopexin (+32%), transferrin (+26%) and IgM (+33%) and the *decreasing* concentrations of albumin (−23%) alpha-1 antitrypsin (−39%) ceruloplasmin (−18%) complement C'3 (−13%) IgG (−26%) and IgA (−29%) were statistically non-significant. Compared to the values of the first week after uncomplicated renal transplantation the corresponding concentrations of hemopexin ($p<0.01$) and complement C'3 ($p<0.05$) were decreased, while beta-lipoprotein in the six patients was elevated before the rejection episode ($p<0.01$).

Table II. *The doses of prednisone and Imurel in the ten patients before, during and after the rejection episodes (mg/day)*

	Before		During progression		During remission		After	
Pat. no.	Prednisone	Imurel	Prednisone	Imurel	Prednisone	Imurel	Prednisone	Imurel
2	10	100	20	100	20	100	15	100
3	0	100	100	50	100	50	80	50
5	30	100	150	100	100	100	60	75
7	30	0	150	50	125	50	40	50
14	15	100	30	100	100	150	60	125
17	30	705	30	100	200	100	150	100
22	0	200	80	100	80	100	60	125
23	0	200	150	75	125	100	90	100
31	100	150	550	200	550	150	300	150
34	40	150	100	200	150	200	90	150

During late rejection in four patients no change could be statistically stated. *Increasing* serum concentrations of haptoglobin (+40%), pseudocholinesterase (+23%), alpha-2-glycoprotein (+33%) and IgM (+25%), and *decreasing* concentrations for prealbumin (−17%) alpha-1 lipoprotein (−19%) albumin (−18%), easily precipitable glycoprotein (−13%), Gc protein (−13%), alpha-2 HS glycoprotein (−43%), ceruloplasmin (−20%) alpha-2-macroglobulin (−24%) transferrin (−9%) IgG (−13%) and IgA (−18%) were statistically non-significant.

Table III. *The mean concentration and S.D for 21 serum proteins before early and late rejection episodes compared with those earlier published (11) at the same time after transplantation. The values for 21 controls are included*

au arbitrary units

	Values expressed in	Log. transformation	1st week after ATR		Before early rejection		3rd month after ATR		Before late rejection		Controls	
			Mean	S.D	Mean	S.D.	Mean	S.D	Mean	S.D.	Mean	S.D
Prealbumin	g	No	0.26	0.11	0.24	0.08	0.54	0.08	0.42	0.18	0.24	0.07
Orosomucoid	g	Yes	0.404	0.092	0.320	0.139	0.170	0.030	0.200	0.114	−0.125	0.078
α_1-lipoprot.	au	No	131	42	121	32	201	18	172	42	99	28
Albumin	g	No	33.3	5.2	34.8	6.7	42.4	0.9	39.8	4.9	47.1	4.5
α_1-antitrypsin	g	No	3.41	0.86	2.91	0.75	1.56	0.49	1.84	0.28	1.65	0.30
Ein. prec. glycop.	au	No	109	28	101	24	118	15	115	28	94	12
α_{1T}-glycoprot.	au	Yes	2.498	0.116	2.406	0.258	2.197	0.092	2.275	0.070	2.018	0.072
Gc-protein	g	No	0.32	0.07	0.31	0.08	0.32	0.04	0.24	0.04	0.27	0.06
α_2-HS-glycoprot.	g	No	0.36	0.18	0.38	0.22	0.30	0.03	0.34	0.19	0.63	0.16
Haptoglobin	g	Yes	0.648	0.141	0.342	0.569	0.546	0.052	0.336	0.171	0.192	0.187
Ceruloplasmin	g	Yes	0.598	0.174	−0.794	0.153	−0.897	0.071	−0.860	0.107	−0.597	0.210
α_2-macroglobulin	g	No	2.44	1.18	2.31	0.44	2.44	0.59	2.32	0.59	3.00	0.81
Pseud. cholest.	mg	No	13	2	13	2	11	2	8	2	9	2
α_2-glycoprotein	au	No	101	34	87	47	151	11	100	18*	85	11
Hemopexin	g	Yes	−0.187	0.205	−0.037	0.146*	0.115	0.011	0.057	0.122	−0.108	0.063
Complement C'3	g	No	0.92	0.47	0.69	0.34	0.84	0.22	0.57	0.14	0.77	0.15
β-lipoprotein	au	No	106	57	153	44*	126	10	138	20	156	33
Transferrin	g	Yes	0.015	0.254	0.094	0.167	0.308	0.046	0.156	0.155	0.366	0.090
IgG	g	Yes	0.849	0.187	0.784	0.086	0.872	0.092	0.979	0.151	1.031	0.098
IgA	g	Yes	0.123	0.321	0.105	0.324	−0.232	0.143	−0.089	0.270	0.213	0.204
IgM	g	Yes	−0.691	0.179	−0.670	0.214	−0.325	0.138	−0.308	0.025	−0.210	0.235
Number			5		6		3		4		21	

P values: <0.01, 0.01–0.05

Fig. 2. The serum levels of 21 serum proteins during an early and late rejection in a 44-year-old woman.

Compared to the values of the third month after uncomplicated renal transplantation (Table III) the concentration of alpha-2-glycoprotein was reduced ($p < 0.01$). During all rejection episodes in the ten patients the fall in albumin and Gc protein concentrations were statistically significant ($0.01 < p < 0.05$).

Fig. 2 shows the serum concentrations of 21 proteins during early and late acute rejection in a 44-year-old woman (no. 31).

DISCUSSION

The present study shows a significant fall in the concentration of albumin and Gc protein during acute rejection after renal transplantation. A similar change occurs during other complications following renal transplantation (7) and is possibly due to impairment of the synthetic function of the liver (7). The remaining 19 serum proteins studied were not significantly changed during the rejection episodes.

A different pattern was found in early and late rejection (Figs. 1 and 2 and Table III). During *early rejections* the electrophoretic pattern seemed unstable, probably due to the recent transplantation, the abolished uremic condition and the commencement of immunosuppressive treatment. *Before* clinical signs of rejection, hemopexin and complement C'3 were decreased and beta-lipoprotein increased compared with five transplanted patients with uneventful course. Haptoglobin was low before rejection but the change was insignificantly due to the high variation of the values. *During* the early rejections prealbumin increased, but a similar change occurs unrelated to rejection during the first week after transplantation (7).

At the commencement of *late rejection* the electrophoretic pattern seemed more stable. *Before* clinical signs of rejection the concentration of alpha-2-glycoprotein was low compared with that in three patients with an uneventful course (Table III). The function of this low molecular protein is unknown but reduced concentrations were also found during acute hepatitis and other complications following renal transplantation (7). The low concentrations of haptoglobin hemopexin and complement C'3 before the late rejection were not statistically significant. Likewise the small changes of serum protein concentrations *during* late rejection were statistically non-significant.

The subnormal value of complement C'3 before clinical signs of rejection, and the further decrease later in the course of rejection point to a complement-consuming antigen-antibody reaction. Also a slight fall in IgG and IgA in some patients accounts for such reactions taking place, and obviously in the kidney graft.

Statistical analysis of the present small material has not revealed with significance any serum protein changes which occur in characteristic association with acute rejection of a renal allograft. More conclusive results may be obtained by examination of a larger case material, but it will further be necessary to investigate the influence of immunosuppressive treatment and the metabolic role of the kidney transplant in the previously uremic organism. Such studies will presuppose determinations of production, distribution, degradation and loss of the individual proteins by means of purified labelled preparations.

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PACEMAKER SOUND DUE TO STIMULATION OF THORAX MUSCLES BY CARDIAC PACEMAKERS

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Abstract. Patients with pacemaker sound due to stimulation of thoracic muscles from cardiac pacemakers are described. The pacemaker sound can be recorded immediately after the pacemaker impulse. The contractions of the intercostal muscles can be recorded on an apex cardiogram. The intensity of the pacemaker sound will in different patients vary from very weak to equal to the loudest heart sound. In some patients pacemaker sound is intermittent, often related to respiration or posture. The incidence in 38 consecutive patients on unipolar endocardial pacing was 26%. Pacemaker sound may indicate current leak or electrode perforation in patients on bipolar endocardial pacing, although the pacemakers seem to function well in the majority of such patients. The disappearance of previously permanent pacemaker sound may indicate battery exhaustion. The muscular contractions associated with pacemaker sound will be felt as unpleasant by some patients.

In patients on cardiac pacing an *extra sound may* sometimes be heard during auscultation of the heart. The sound appears synchronously with the pacemaker impulse and, when the heart is stimulated by the pacemaker will appear as an extra first heart sound.

It has been demonstrated that this extra "pacemaker sound" originates from contractions of the thorax muscles, triggered by the pacemaker impulse (2, 3, 6). Pacemaker sound has been observed in patients on epicardial, as well as on endocardial, pacing and with unipolar as well as bipolar electrodes. In patients with bipolar electrodes it has been suggested to indicate current leak (4). The incidence has been reported to be from 25%–80% (4, 8).

The present report deals with incidence and some clinical observations associated with pacemaker sound.

MATERIAL AND METHODS

After pacemaker sound had been observed incidentally in four patients on unipolar endocardial pacing, we decided to evaluate the incidence of pacemaker sound in patients treated in this hospital. Forty-seven consecutive non-selected patients admitted for implantation or change of pacemakers were examined with phonocardiogram. Thirty-eight patients were on unipolar endocardial pacing (Cordis pacemakers and electrodes). In these patients the electrodes had been positioned during TV-monitoring with the patients lying on the back. The electrodes were primarily advanced to the right ventricular outflow tract, then pulled back and positioned in the right ventricle. A position in the coronary sinus was excluded by side-to-side TV-screening. One patient had epicardial electrodes and QRS-triggered (Ectocor Cordis) pacemaker, two bipolar Medtronic endocardial electrodes and Medtronic 5841 Demand pacemaker and six had P-synchronous pacemakers (Atricor Cordis) on epicardial electrodes.

RESULTS

The incidence of pacemaker sound appears from Table I. Pacemaker sound was observed in 26% of patients on unipolar endocardial pacing, in 17% on P-synchronous pacing, and in both patients with bipolar Medtronic electrodes. In two patients on unipolar endocardial pacing the sound was not heard, being merely observed on the phonocardiogram.

The character of the pacemaker sound has usually been soft, not very different from the ordinary first heart sound. The intensity has been varying, from the loudest audible sound to very weak. Examples of pacemaker sounds are given in Figs. 1–3.

In two of the four patients observed before the start of the systematic survey the pacemaker

Table I *The incidence of pacemaker sound*

Type of pacing	No. of pats. examined	No of pats. with pacemaker sound
Unipolar endocardial	38	10
Unipolar epicardial	*1*	*0*
Bipolar endocardial	2	2
P-synchronous epicardial	6	1

sound was intense and easily heard during heart auscultation. Both had failing pacemakers, and the pacemaker sound caused diagnostic difficulties. In one patient the pacemaker sound was heard as a late diastolic sound when the pacemaker triggered the heart, but when the pacemaker failed pacemaker sound was *still* heard (Fig. 4) In the other patient the pacemaker sound during long periods occurred late in systole, simulating a systolic click (Fig. 5). These two patients clearly demonstrate that the pacemaker sound is wholly independent of cardiac depolarisation and contraction. In four patients pacemaker sound was intermittent, related to the respiratory phase, or appearing when the patient was lying on the side. One of these patients complained of palpitations when she was lying on the side and therefore preferred to lie on her back. It appeared that a pacemaker sound regularly occurred when the patient had palpitations in the lateral position. Palpitations as well as pacemaker sound disappeared when the patient turned on her back again.

In two patients with Medtronic bipolar electrodes pacemaker sound was observed. Muscular twitching in the thoracic wall has previously been noted in two other patients with Medtronic bipolar electrodes. In these two patients phonocardiograms were not recorded. In none of the *four patients has pacemaker failure due to current leak occurred.*

In one patient with muscular twitchings in the right chest wall adjacent to the indifferent pacemaker electrode, a pacemaker sound was heard and recorded phonocardiographically over the *right pectoralis region (Fig 6).*

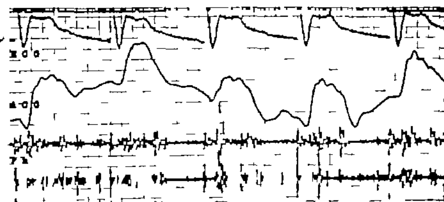


Fig 1 Pacemaker sound in patient on fixed rate pacing. The sound intensity is low and it is immediately followed by an xtra presystolic ve on the pex cardiogram.

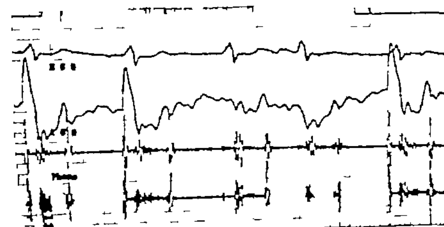


Fig 2 Pacemaker sound in patient with demand pacemaker and bipolar endocardial electrod. The intensity of the sound is high and the consequent wave on the pex cardiogram is marked. Muscular contractions are visible on the chest surface.

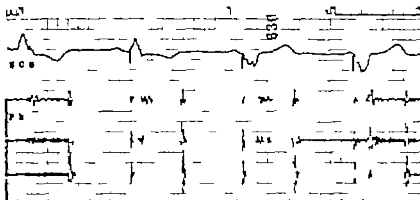


Fig. 3. Pacemaker sound in patient with demand pacemaker and bipolar endocardial electrode. A pacemaker sound occurs before the third and fourth beat which are pacemaker-induced, and before the second beat which is fusion beat, but not before the first sinus beat.

DISCUSSION

Pacemaker sound of the type described has proved to be due to pacemaker-induced contractions of the thorax muscles (2, 3, 6). The time interval from the pacemaker impulse to the pacemaker sound corresponds to the electromechanical interval of skeletal muscles. The muscular contractions have been demonstrated on apex cardiograms. A similar pacemaker sound may be observed over the right chest or the abdominal wall when the respective muscle groups are stimulated by an indifferent pacemaker electrode. In patients with interference between the pacemaker rhythm and the intrinsic heart rhythm, pacemaker sound has been demonstrated during systole and therefore must be independent of heart depolarisation or contraction.

Another type of pacemaker-induced sound has also been described. It appears only during systole, as a scratchy squeaky high-pitched murmur and is probably due to mechanical interference of the catheter electrode with the tricuspid valves (7). This type of the sound is related to the intracardial catheter as such, and not to the pacemaker impulse.

The occurrence of pacemaker sound due to muscular stimulation is probably related to the position of the intracardial electrode. The current density in the skeletal muscles has to exceed the threshold value for muscular stimulation and therefore the distance from the pacemaker electrode to the thorax wall cannot be too great. The muscular contractions may subside if the thorax wall is moved away from the heart, as may be demonstrated in patients with inconstant pacemaker sound, related to posture or respiration. A pacemaker sound will probably also disappear if the current output from the pacemaker is reduced. In patients in whom a permanent pacemaker sound has been recorded, its disappearance may therefore be an indication of battery exhaustion.

In different series of pacemaker-treated patients the incidence of pacemaker sound will vary probably depending on the type of pacemaker electrodes used and the intracardial position attempted. A high incidence has to be expected when an anterior position is chosen. In the present series the electrodes were positioned after withdrawal from the right ventricle outflow tract, with the patient lying on his back. The electrodes



Fig. 4. Pacemaker sound in patient with an inconstantly failing pacemaker. After the first, second and fourth pacemaker impulses triggering occurs and normal heart sound follows, impulse nos. 3 and 5 do not trigger and are therefore not followed by heart sounds.

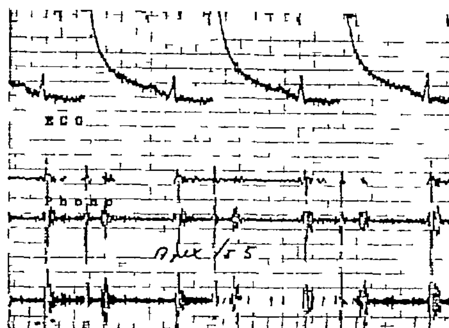


Fig 5 Pacemaker sound during systole in patient with interference between pacemaker and sinus rhythm. In this recording the frequency of the sinus rhythm incidentally is the same as that of the pacemaker rhythm, therefor no pacemaker impulse triggers the heart.

lay in the anterior or middle part of the right ventricle, perhaps toward the inter-ventricular septum, and the incidence of pacemaker sound was not very high, 26 % In patients with epicardial electrodes in the apex region an incidence of 80 % has been reported (8).

In patients with bipolar endocardial electrodes the current density will be high close to the electrodes, in areas lying between the positive and negative poles, and will fall rapidly at a distance from the electrodes. Therefore in these patients the incidence of pacemaker sound will probably be closely related to the distance between the pacemaker electrode and the thorax muscles. If the distance is very short a high incidence may be expected, otherwise the incidence will probably be low It has been postulated that the occurrence of pacemaker sound in a patient with bipolar endocardial electrode is an indication of current leak or of electrode perforation of the right ventricle (4). Our experience, as well as others indicates that pacemakers may function well for a long period in such patients (1 5). Electrode perforation has, however been demonstrated in one case (3) and sudden failure of pacing in . of 14 patients (4). The observation of pacemaker sound in a patient with bipolar endocardial electrode, therefore, warrants close observation with regard to insulation defect with current leak or electrode perforation.

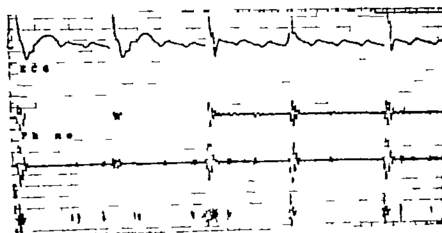


Fig 6 Pacemaker sound over the right thorax, due to muscular stimulation by the indifferent pacemaker electrode.

The diagnosis of pacemaker sound is usually easy if a simultaneous ECG and phonocardiogram are recorded. In patients on atrial synchronous pacing it may be difficult to distinguish between an atrial 4th sound and a pacemaker sound. A pacemaker sound may be disclosed by the recording of muscular contractions on an apex cardiogram, which usually may be separated from an atrial a-wave.

Usually the demonstration of pacemaker sound has little clinical significance. Some patients, however will feel the muscular contractions unpleasant, and describe them as palpitations. The muscular contractions will be felt more easily if they are inconstant or dependent on posture. The reassurance of the extracardial nature of the sensations may relieve the apprehension in some of these patients.

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MALIGNANT LYMPHOGRANULOMATOSIS AND ANTICONVULSANT THERAPY

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Abstract. A case is described of a woman who, after three years of diphenylhydantoin therapy because of epilepsia, developed a rapidly lethal condition which at autopsy proved to have the histopathological picture of malignant lymphogranulomatosis. Reticulum cell hyperplasia of the type which has been described as a complication of hydantoin therapy was also present. Malignant lymphoma in a few patients receiving diphenylhydantoin medication has been described earlier. This is the first such case in which microscopically verified malignant changes in liver, spleen and bone marrow have been reported.

Hydantoin derivates are of great therapeutic value because of their potent anticonvulsant effect. But they have many side-effects. Björnberg and Holst (2) mention gingival hyperplasia, gastrointestinal discomfort, cerebellar dysfunction and a SLE-like syndrome, as well as dermatological symptoms such as hyperpigmentation, hypertrichosis, generalized morbilliform or scarlatiniform exanthema, erythrodermia, Stevens-Johnsons syndrome and toxic epidermolysis. Among the hematological side-effects they mention methemoglobinemia, megaloblastic anemia, granulocytosis, thrombocytopenia and aplastic anemia. Eosinophilia, monocytosis (4) and hemolytic anemia (26) have been described, too, as also atrioventricular block (20) and hyperglycemia (19).

Lymphadenopathy has frequently been described in the last few years as a complication of hydantoin therapy. It is usually combined with other side-effects but occasionally isolated. The clinical picture varies from a silent, local lymph node enlargement to a generalized lymphoreticular engagement with severe malaise. The histological picture may resemble that of malignant reticulosis but most often allows a differential diagnosis. Typical malignant lymphogranulomatosis and lymphosarcoma developing in conjunction with hydantoin therapy have, however, been reported. Such a case is described below together with a review of the literature on hydantoin therapy and lymphadenopathy.

CASE REPORT

A woman born in 1897 sustained a cranial fracture in 1923 with cerebral concussion. She afterwards had headache and fits resembling minor seizures without prodromes or convulsions. From 1957 she attended the Department of Medicine because of these symptoms. In 1967 the EEG was slightly abnormal, in 1968 slightly hyperactive tendon jerks on the left side were noted. Carotid angiography on the right side revealed nothing remarkable. In July 1960 the patient was treated with mephenytoin (Mesantoin, Sandoz, 0.1 g twice daily) for four weeks without any side-effects. In Dec. 1965 diphenylhydantoin (Difhydan, Leo) was started in a dose of 0.1 g three times a day and continued until a few days before death. In 1967 phenobarbital, 0.1 g in the evening, was added, and in Nov. 1968 sulthiame (Ospolot, Bayer), 0.2 g two to three times a day. A certain improvement was noted.

At Christmas 1968 the patient became increasingly tired. She had a dry cough and began to lose weight. Her condition deteriorated in 1969 and she began to have spells of fever. The intervals and severeness are not known. In July 1969 exertional dyspnea was noted.

The patient was admitted to the Medical Clinic on July 24, 1969. She appeared extremely fatigued, emaciated and somewhat hyperpigmented and jaundiced. There was no palpable enlargement of liver, spleen or lymph nodes.

Laboratory findings

Hb 11.3–10.9 g/100 ml. R.B.C. 3.5 mill./µl. W.B.C. 7 200–10 700/µl. Differential count 74–80% bands and segmented neutrophils, 0–0.5% eosinophils, 0–1 basophils, 21–13% lymphocytes, 4.5–5.5% monocytes. Reticulocyte count 20 000/µl. Platelets 272 000/µl. Serum bilirubin 35–18 mg/l with positive direct reaction. SGPT 29–23 Karmen U., SGOT 30–25 Karmen U., serum alkaline phosphatase 15–13 Bosch & Busch U., serum gamma-glutamyl transpeptidase 645 U. (normal 15–80), serum

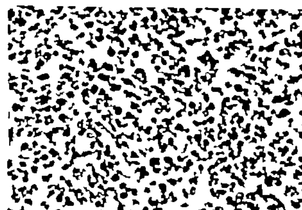


Fig. 1. Lymph node from axilla. Benign reticulum cell hyperplasia. H.E. × 250.

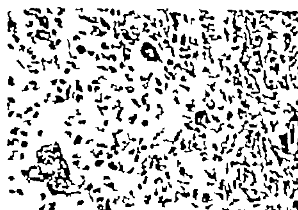


Fig. 3. Lymph node from liver hilus with tendency to fibrillar necrosis. Note Reed-Sternberg cell. H.E. × 300.

amylase 34 U and serum lactate dehydrogenase 500 Wróblewski U with slight elevations of the isoenzyme fractions LDH, and, less pronounced, LDH and LDH. Serum fetum negative. Owren prothrombin-proconvertin activity 62–81%. Other detailed coagulation analyses revealed only moderately raised titer of fibrinolytic split products. Blood cultures negative. The urine showed pathological sediment and culture gave growth of Proteus. The tests for blood in the stools were negative. Extensive serology revealed nothing abnormal. ESR 19–12 mm 1 h. Serum protein was 55–49 g/l. Agarose gel electrophoresis and quantitative plasma protein determination showed pattern of inflammatory reaction. Total antitrypsin, orosomucoid, haptoglobin and ceruloplasmin raised. Albumin reduced to 31 g/l. Marked hypogammaglobulinemia with IgG g/l, IgA 0.5 g/l and IgM 0.2 g/l.

Needle biopsy of the liver showed many lymphocytes in the portal zones but no diagnosis was possible. Bone marrow examination was not helpful. X-ray of chest normal. Spleen and liver of normal size at X-ray examination.

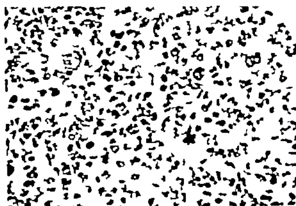


Fig. 2. Lymph node from liver hilus. Malignant lymphogranulomatosis with Reed-Sternberg cells and atypical mitosis (slightly out of focus). H.E. × 220.

Clinical course

The patient's condition rapidly worsened. She continuously had fever (38°C) and peaks of 40°C at interval of about 48 hours. She finally became disoriented and comatose. Antibiotics had no effect. Some days before death treatment with salicylates was started but was stopped on the following day because of the appearance of generalized morbilliform exanthema. This reaction raised the suspicion of relationship between the patient's illness and her anticonvulsant therapy, which at that time, however, was not regularly maintained because of her poor general condition. She died on Aug. 12.

Clinical diagnosis

The clinical picture was considered consistent with diagnosis of malignant reticulosis.

Autopsy findings

Extensive bronchopneumonia. Brain: no abnormality. There was calcified plaque in the leptomeninges of the left cerebral hemisphere. Liver of normal appearance. Spleen enlarged (780 g), cut surface homogenous. Moderately enlarged, discrete lymph nodes, but no gross signs of tumor.

The microscopic picture of the nodes varied but none was of really normal appearance. In the iliac groins and along the aorta and subclavian arteries the architecture of the nodes was preserved with wide sinuses filled with macrophages, many of which showed phagocytosis of erythrocytes. In the parenchyma there were diffuse foci of benign-looking reticulum cell hyperplasia with some mitoses, but without prominent atypia (Fig. 1). Very few plasma cells or eosinophils. The nodes in the hilus of the liver and the lungs contained similar foci, but also foci containing very polymorphous tissue with bizarre reticulum cells and even typical Reed-Sternberg cells with mirror-image nuclei, giant nucleoli and many mitoses, some grossly abnormal (Fig. 2).

This kind of tissue destroyed the nodal architecture in many areas but did not infiltrate the capsules. Some small foci of fibrillar necroses (Fig. 3) but no fibrous. Some plasma cells and eosinophils were found in such

areas. In the portal zones of the liver there were numerous foci of atypical reticulum cells (Fig. 4). The spleen was diffusely infiltrated with atypical reticulum cells of rather monomorphous appearance but showed no typical Reed-Sternberg cells. This tissue resembled reticulum cell sarcoma (Fig. 5). There was slight tendency to necrosis but no fibrosis. The bone marrow contained numerous small foci of this kind.

DISCUSSION

In 1959 Saltzstein and Ackerman (2) reported seven cases of hydantoin-induced lymphadenopathy together with a review of the literature. It was demonstrated that lymphadenopathy had been seen already in the 1920s when Nirvanol (5-ethyl-5-phenyl-hydantoin) was introduced in the treatment of Sydenham's chorea. There are no reports of the histology of these lesions. In 1940 Cooper and Burrows (5) described a patient who developed a maculous rash with fever and cervical lymphadenomegaly after seven days' treatment with diphenylhydantoin. The symptoms disappeared on withdrawal of therapy but appeared again on reinstitution. Seventy-five cases were afterwards reported in American and European literature, the latest of which was Lindqvist's in 1957 (15), before Saltzstein and Ackerman in 1959 raised the total figure to 82. Summing up, many different hydantoins and trimethadione, too, were incriminated. In the majority of cases the reaction appeared within one month. Only four had been treated for more than four months. Another common feature was that the lymph node enlargement disappeared within some weeks of withdrawal of the drug but soon returned if medication was resumed. Most cases had lymph node enlargement in combination with other signs, often as a characteristic triad of fever, rash and cervical lymphadenopathy and frequently also eosinophilia. The cervical nodes were not the only ones affected, however. Hepatomegaly and/or splenomegaly was noted in eleven cases. In some cases more severe complications were a dominating feature, such as bone marrow suppression, liver damage with icterus, and SLE-like pictures. There were no significant differences in age or sex distribution. The histological picture of the nodes had sometimes been reported and was described as a varying degree of effacement of nodal structure and a pleomorphic cellular outfit with reticulum cell hyperplasia, many mitoses and eosinophils, neutrophils and plasma cells. Necrosis, often hemorrhagic, had sometimes been noted. Nuclear fragmentation and phagocytosis were common findings. The picture often resembled Hodgkin's disease, but, according to Saltzstein and Ackerman, Reed-Sternberg cells are never seen and this should be noted as a differentiation from Hodgkin's disease. Reticulum cell sarcoma-like tissue had been described in some cases, but the uniformity of cell type usually seen in sarcoma was absent owing to the admixture of many inflammatory cells, especially eosinophils. After Saltzstein and Ackerman's paper many more reports appeared (1, 2, 3, 9, 14, 23, 27). These cases are similar to the older ones in most respects.

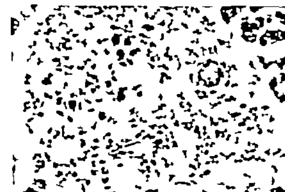


Fig. 4. Liver. Portal zone with lymphocytes and atypical reticulum cells. H-E, ×250.

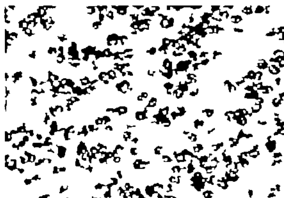


Fig. 5. Spleen. Diffuse, atypical reticulum cell hyperplasia with many mitoses. H-E, ×300.

In recent years malignant reticulosis and lymphoma have been described in connection with hydantoin therapy. One of the patients reported

by Saltzstein and Ackerman in 1959 died in 1960 from a generalized malignant lymphoma. In 1956 this patient had a rash and cervical lymphadenomegaly after six years of treatment with diphenylhydantoin. At the time of death he had been without hydantoins for some years (10). Hyman and Sommers (12) in 1966 described three cases of Hodgkin's disease and three cases of lymphosarcoma that developed during diphenylhydantoin therapy, which had been given for 2–17 years. The histological picture was described as diagnostic of the respective conditions. Two of the patients with lymphosarcoma died, one of them from the disease despite withdrawal of therapy, the other from assumed cerebral arteriosclerosis. Neither of these patients was autopsied. The other four patients received radiotherapy or cytostatic compounds with good effect and could, with but one exception, continue to take hydantoins. Suspect malignant lymphoma in patients receiving hydantoins has also been reported by Rosenfeld et al. (21) and Doyle and Hellström (6). At the time of report their patients were well, the lymphadenopathy having regressed after discontinuation of therapy, and, in the case reported by Doyle and Hellström, administration of nitrogen mustard. In 1968 Gams et al. (7) reported a patient who developed a lymphadenopathy resembling a malignant lymphoma. On withdrawal of hydantoins all symptoms and signs disappeared, but more than a year later a true malignant lymphoma appeared, which led to the death of the patient. The tumor was verified at autopsy. Gams et al. consider it impossible to differentiate microscopically between drug reaction and neoplasia in borderline cases.

Our case was clinically regarded as malignant reticulosis. Autopsy confirmed malignant lymphoma localized mainly to the liver and lymph nodes in the liver hilus. It is noteworthy that also changes consistent with those described after hydantoin therapy were present. They are primarily characterized by benign-looking reticulum cell hyperplasia. Besides this, we found the lymph nodes and the liver to contain tissue of malignant histological appearance with the characteristics of malignant lymphogranulomatosis. In the spleen and the bone marrow there were changes strongly suggesting reticulum cell sarcoma. Some lymph nodes contained hyperplastic as well as evidently malignant foci and fields with an intermediate picture. A search of the literature failed to reveal any reports of a malignant lymphoma during hydantoin therapy and involving the liver, the spleen or the bone marrow. It may be noted that diphenylhydantoin is metabolized mainly in the liver (27).

Cases of assumed drug-induced malignancy should be regarded with much scepticism. In this patient the clinical course and the microscopic picture gave clear evidence of a malignant disease. It is, of course, impossible to know with certainty the relation, if any, with the drugs given. A causal relationship with the diphenylhydantoin may be suspected because of the occurrence of changes of the type often induced by the drug and malignant tissue in close proximity, and the intermediate picture seen in some areas. Also earlier reports of co-occurrence seem to favor a causal connexion. On the other hand, hydantoins are widely used anticonvulsants and the reports of co-existence are only few. All of the patients who developed malignant lymphoma seem to have received diphenylhydantoin. In our patient the deterioration occurred soon after treatment with sulthiame, which is not a hydantoin derivative. We have not found any reports on lymphadenopathy in conjunction with this drug.

The mechanism of the induction of lymphoreticular hyperplasia is unknown. Animal experiments have been made by Kaslaris (13), who induced lymphadenomegaly in a cat by oral administration of mephenytoin in large doses. Lymph node enlargement was observed already on the 5th day and the animal died on the 31st day after severe toxic side-effects. The histology of the nodes in that animal showed reticulum cell hyperplasia without cytological malignancy. A more extensive study was made by De Snijjer et al. (28), who administered mephenytoin and trimethadione to mice. They found "a marked hypertrophy of the lymphoid formations and in some cases a slight evolutive hyperplasia, which is not exactly atypical". A certain histiocytic reaction was noted in almost all the animals. No reports of experimental induction of malignant tumors are available.

There is clinical and experimental evidence of hydantoins stimulating cell growth. The gingival hyperplasia is well known. Stimulation of wound healing (24) and increase of dermal collagen (11) have also been reported. Shafer (25) found an increased rate of growth in cultures of human fibroblasts after addition of diphenylhydantoin as

well as a certain stimulation of He-La cells and certain other tumor cultures. The possibility of induction of malignant reticulosis by a long acting growth-stimulant cannot be excluded. It should also be borne in mind that a dormant malignancy might be activated by a compound more or less specifically acting as a stimulant to the tissue of origin. The exanthema and the eosinophilia, which may occur simultaneously seem to favor the assumption that the lymphadenopathy is allergic or idiosyncratic in nature. This may also explain why only a few of those treated are affected. Malignancy seems to develop only after years of continuous therapy In such cases a low grade and subclinical reaction may have existed for a long time before malignant transformation occurs. A prolonged stimulation of the reticulo-endothelial system has been suggested as a possible etiology in cases of plasma cell dyscrasias (13) Hydantoins have been involved, too, in cases resembling plasma cell dyscrasias. Branco and Gander s patient (3), a woman of 30 developed malaise, fever eosinophilia and rash some days after institution of mesantoin therapy A little later on, she developed a lymphadenopathy with a histological picture regarded as Hodgkin s disease with Reed-Sternberg-like cells and many atypical plasma cells in the node. At the same time hyperproteinemia of 120 g/l with 66.7% of gamma globulin in a narrow peak was detected. One month and a half after withdrawal of mesantoin the patient was well and the electrophoresis was almost normal. The first pattern had the appearance of "Kahler's or Waldenström's disease No ultracentrifugation is reported. Other cases of plasma cell dyscrasia and hydantoin reaction have been reported but the time relationships are difficult to judge. Saltzstein and Ackerman s patient no. 3 had bone marrow plasmocytosis after the drug reaction and later died of multiple myeloma. Hyman and Sommers (12) reported case (no. 4) with lymphosarcoma and a small, homogenous gamma globulin peak after hydantoin therapy The patient of Olmer et al. (no. 4) (16) developed a plasma cell leukemia-like picture leading to death. No electrophoresis was reported. Cases with hypogammaglobulinemia and hydantoin reaction have been described earlier both malignant" (12) and of the reversible type (2). We do not know for how long our patient had had hypogammaglobulinemia. The condition may have been secondary to the lymphoma or may have developed without any relation to other disease. On the other hand a chemical compound might possibly induce such a state in predisposed individuals by interference with lymphoreticular tissue. This may lead to malignant proliferation of cells otherwise held in check by immunological mechanisms. Lymphoma seems to be common in both congenital and acquired" hypogammaglobulinemia (8 17). The SLE-like syndromes may have a similar background through the activity of potentially autodestructive mutant clones, otherwise repressed. Whatever the mechanism of interaction may be, there seems to be a risk of interference with the immunologically active cell systems and the patients treated should be watched for such interference.

Addendum. W are presently observing another patient with lymphomatous disorder in which relationship to diphenylhydantoin therapy may be suspected. This woman, born in 1909 developed seizures after an operation for osteomyelitis in the middle ear in 1931. Diphenylhydantoin (0.1 g three times day) was started in 1962 and the seizures disappeared. In 1967 splenomegaly was diagnosed. The spleen presently reaches three finger breadths below the umbilicus. A lymphadenopathy has also developed. A biopsy of node in 1968 showed effacement of structure by small lymphocytes intermingled with many large, morphologically benign reticulum cells. The picture is consistent with ell differentiated lymphocytic lymphosarcoma. Aspiration biopsy of the spleen gave rich smear dominated by moderately well differentiated lymphocytes but also some reticulum cells and plasma cells. A representative bone marrow smear contained 30% lymphocytes, some atypical. W.B.C. around 4 000/µl with 40–60% lymphocytes. Plasma protein analyses revealed an inflammatory reaction and polyclonal hypergammaglobulinemia with IgG 24–18 g l, IgA 3–3.5 g l and IgM 2.1 g/l. Kappa light chains have occurred in considerable amount in the urine.

Some atypical features of this case have raised the suspicion of relationship to the anticonvulsant therapy which has recently been stopped. No conclusions can be drawn as yet concerning possible effects of this.

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RETURN OF HUMAN LEUKEMIC MYELOBLASTS FROM BLOOD TO BONE MARROW

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Abstract. A patient with acute myeloblastic leukemia received a single intravenous injection of Methotrexate, followed 16 hours later by vincristine. The total blast count remained stable for 16 hours after Methotrexate, then declined moderately and from 24 to 48 hours after Methotrexate fell exponentially with a half-time of approximately 4 hours to 1/50 of the original value. This is not easily explained from leukemic cell kill alone, and it is suggested that such a rapid and massive reduction in blood blast count may in part be due to an anatomical redistribution of blast cells. That blast cells do return from the blood to the marrow was shown by studies of the fate of labeled cells: immediately prior to Methotrexate, blast cells from the blood were labeled with ^{3}H-cytidine and then rapidly reinfused. Serial blood and marrow smears were obtained and autoradiographed. The number of labeled blasts in the blood closely paralleled the total number of blasts in the blood throughout the 6-day study period. Nine bone marrows were obtained between 6 and 96 hours after autotransfusion, in all samples significant numbers of labeled blasts were found in the marrow which could not be accounted for by blood contamination. An attempt to demonstrate that labeled cells, which return to the marrow will resume cell division was unsuccessful.

In previous studies of leukemic cell kinetics in man, utilizing ^{3}H-thymidine labeling in vivo, it was demonstrated that leukemic cells are lost from the blood in a random fashion with a half time of about 24 hours (8). This has been confirmed in other ^{3}H-thymidine studies and by observations of autotransfused blast cell from the peripheral blood which had been labeled with ^{3}H-uridine in vitro (2). The subsequent fate of blood blasts is not known, except that it has been stated that they apparently do not return to the marrow to any appreciable extent (2).

The present report deals with the kinetics of blast cells in the blood during antileukemic therapy: evidence is presented that—at least under these conditions—leukemic myeloblasts return from the blood to the marrow.

MATERIAL AND METHODS

The patient was a 29-year-old woman with acute myeloid leukemia diagnosed 12 months prior to the study. Initially she was treated with prednisone, vincristine, Methotrexate, and 6-mercaptopurine and went into a five months' clinical remission. During the subsequent relapse she was again intermittently treated with vincristine, Methotrexate, prednisone, and 6-mercaptopurine. At the time of the study she had for several months been on prednisone, 15 mg daily: this was continued during the study. 6-mercaptopurine was the last cytostatic agent given, and had been discontinued four weeks earlier. Although the blast cell count in the blood had been rising for some time, it remained rather stable for the last five days prior to the study (WBC 78 000–64 000–73 000) with 88–92% leukemic cells (blast cells + a few per cent promyelocytes).

Laboratory procedures

On the day of the study 500 ml of blood were drawn into a Fenwal bag containing ACD solution and 1 mCi of ^{3}H-cytidine (sp. a. 5.0 Ci/mM, New England Nuclear Corp.) and incubated for two hours at 37°C. Without further manipulation the blood was then autotransfused within 20 min. Samples of blood and bone marrow were withdrawn at intervals as indicated in Fig. 1 and Table I. Blast cell counts were determined from hemocytometer leukocyte counts and differential counts of 200 cells in the blood smears; the few per cent of abnormal promyelocytes present were pooled with the myeloblasts. To facilitate the counting of autoradiographs, cell concentrates were prepared from each blood sample by mixing 3.5 ml of blood with 1 ml of dextran (6% in 0.7% NaCl); after sedimentation, smears were made from the buffy coat, in some instances after slight centrifugation. In vitro labeling of blood and bone marrow with ^{3}H-thymidine was done by incubating about 0.5 ml of marrow or 3.5 ml of blood with 2 μCi of ^{3}H-thymidine

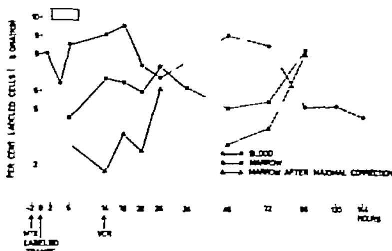


Fig. 1. Percentage of labeled cells in blood and bone marrow after autotransfusion of leukemic blast cells labeled in vitro with ³H-cytidine. *MTX*, Methotrexate. *VCR*, vincristine. "Marrow after maximal correction" refers to the percentage of labeled cells in the marrow after correction for upper limit blood contamination (explained in text).

(sp. a. 1.9 Ci mM, New England Nuclear Corp.) for one hour at 37°C. EDTA was used as an anticoagulant and, in the case of blood, dextran was added as just described.

For autoradiography marrow and buffy coat smears fixed in methanol were dipped in Kodak NTB- emulsion 1 exposed for 30 days (³H-cytidine labeling) or 7 days thymidine labeling) at 4°C, developed and stained with The percentage of labeled blast cells (> 5 grains) determined by counting 3 000 blast cells in each smear. Mean grain counts of cytidine-labeled cells were determined from 25 labeled cells. Mitotic indices are based on at least 3 000 blast cells.

As described later fair numbers of labeled cells were observed in the marrow smears. T ensure that this was not solely explained by admixture of blood with its content of labeled cells to the marrow smears, the following method was used: in corresponding blood and marrow smears, the number of blast cells in 30 consecutive, randomly chosen fields of sion (the photography frame of a Zeiss photomicroscope at 400) was recorded. T correct for differences in smear thickness, the number f red cells in well-defined fraction of each field was also counted. From these data and the percentage of labeled cells in the blood one can calculate the maximum number of labeled cells in the marrow smears which can possibly be accounted for by blood contamination of the

Table I. *Distribution of auto-infused H-cytidine labeled blast cells in blood and bone marrow and cytokinetic parameters of the blast cells*

	³H-cytidine labeled blast cells				³H-thymidine labeled blast cells (%)		Blast cells in mitoses ( )	
	Per cent		Mean grain count					
	Blood	Marrow	Blood	Marrow	Blood	Marrow	Blood	Marrow
Just before Methotrexate	—	—	—	—	0.9	9.9	0	0.7
Hours after autotransfusion of ³H-cytidine labeled cells								
1	8.0	—	54	—	—	—	—	—
4	6.4	—	69	—	—	—	—	—
6	8.5	4.6	55	40	—	19.4	—	0.2
14	9.1	6.7	50	61	—	18.5	—	0.1
18	9.6	6.6	40	50	—	18.1	—	0.5
22	7.4	8.0	49	44	—	—	—	—
26	6.8	7.5	47	51	—	18.1	—	3.7
34	—	6.3	—	55	—	—	—	—
46	9.1	5.1	40	49	—	—	—	—
72	8.5	5.5	25	41	—	12.4	—	—
96	5.2	8.1	30	28	—	13.0	—	—
120	5.3	—	23	—	—	—	—	—
144	4.6	—	4	—	—	—	—	—

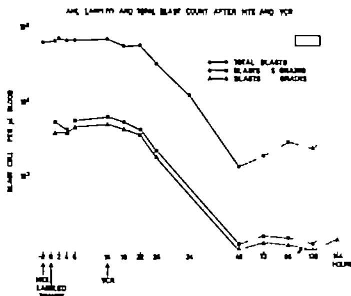


Fig 2. Blood concentration of total blasts and labeled blasts after intravenous Methotrexate (*MTX*) and vincristine (*VCR*).

marrow. This is best seen from an example: with 5% labeled blasts in the marrow and a total of 100 blasts/400 red cells there will be 1.25 labeled blasts/100 red cells in the marrow. If at the same time there are 8% labeled cells in the blood and a total of 40 blasts/500 red cells, there will be 0.64 labeled blasts/100 red cells in the blood. This is the maximum number of labeled cells/100 red cells which possible admixture of peripheral blood could contribute to a simultaneous marrow smear. This figure will be referred to as "upper limit blood contamination". Labeled blasts in marrow smears in excess of this figure must represent cells in the bone marrow proper: in the example chosen it would be 1.25–0.64 = 0.61/100 red cells, i.e. 49% of the labeled blasts in the marrow smears. From the method of calculation it will be apparent that this is a lower limit estimate of labeled *bona fide* marrow blasts.

Therapeutic procedures

Immediately after the 500 ml of blood had been drawn for labeling, 30 mg of Methotrexate was given intravenously. Sixteen hours later, i.e. 14 hours after start of the autotransfusion, 4 mg of vincristine was injected intravenously. The patient's body weight was 54 kg.

RESULTS

The Fenwal bag contained a total of 3.75 × 10 blast cells, of which 55.9% were labeled (> 5 grains) corresponding to a total of 2.1×10^{1} labeled cells. The patient's estimated blood volume was 3.8 l, and from this and the blast cell count the total number of circulating blast cells was calculated to be 25.5×10. In case of 100% recovery of the labeled cells the expected concentration of labeled cells in the blood after the reinfusion would be $2.1/25.5 \times 100 = 8.2\%$. Fig. 1 and Table I show the percentage of labeled cells in blood and bone marrow as a function of time. It is seen that one hour after the reinfusion 8% of the blood blasts were labeled. Fig. 1 also includes data on the percentage of labeled marrow cells after maximal correction, i.e. after subtraction of the "upper limit contamination" with blood blasts as defined in the preceding section. It is seen that, at all points studied, labeled cells were found in the marrow. Table I also includes ^{3}H-thymidine labeling data. Just before the study 9.9% of marrow blasts and only 0.9% of blood blasts were labeled. Eight hours after Methotrexate, the percentage of ^{3}H-thymidine labeled marrow blast cells had doubled; this high level was maintained during the subsequent 20 hours and then declined moderately on the third and fourth day.

Fig. 2 shows the changes in the total blast cell count and total labeled blast cell count in the blood. From the time of Methotrexate injection there is a shoulder lasting for 16 hours; then follows a moderate decline, and at 24 hours a precipitous fall sets in with a half time of about 4 hours: the nadir is reached 48 hours after Methotrexate. Subsequently the blast count again rises slowly. The labeled blasts rather closely follow the curve of total blasts.

In the bone marrow smears labeled mitotic fig

ures were looked for Twelve hours after vincristine (26 hours after reinfusion of the labeled cells) mitoses were numerous. Out of .93 mitoses 41 were slightly labeled (6–11 grains) at the same time the mean grain count of labeled interphase cells in the marrow was 51 In later bone marrow samples (34 46, 72 and 96 hours after reinfusion), mitotic figures were extremely scarce at these points of time a total of 40 mitoses were seen, of which 12 were slightly labeled (6–11 grains). The mean grain count of labeled interphase cells in the marrow at these points of time ranged from 55 to 28, as seen from Table I. In bone marrow aspirates 14 18 and 22 hours after reinfusion, 4 out of 50 mitoses were marginally labeled (6–7 grains).

DISCUSSION

1 *Decline in peripheral blast count*

Kinetically leukemic blast cells are not uniform some are actively proliferating others are non-proliferating (9). However recent evidence (3 5 11) suggests that non-proliferating cells may at least in part resume proliferation hence quies- cells may be a better term. The potential of these quiescent cells (Q-cells) is not yet well defined they may include cells in a long G_1 phase, G_0 cells, and end stage cells. At diagnosis Q-cells constitute 65–85% of blast cells in the marrow and represent an even higher fraction of blast cells in the blood (9) The pool of Q-cells is fed from the actively proliferating pool. It has been calculated (9) that the transit time through the marrow Q-cell pool may vary between two days and two weeks.

Several antileukemic drugs, e.g Methotrexate and vincristine, act primarily on proliferating cells (7). These drugs may therefore be expected to severely curtail the cell flux from the actively proliferating to the quiescent marrow pool. However from the long lifespan of Q-cells one would predict a slow decrease in the size of this pool even if the influx from the actively proliferating cells had been completely stopped, since blast cells in the blood are predominantly of the quiescent type they would be expected to decline slowly after chemotherapy which interferes with blast cell proliferation. However when frequent counts are carried out after single dose chemotherapy it is apparent that the blood blast count often falls rapidly This is illustrated by Fig. 2, which shows that the blood blast count remained constant for about 20 hours after initiation of cytostatic therapy subsequently the count fell exponentially ($T/_{\frac{1}{2}}$ hours about 4 hours) down to 1/50 of the pretreatment level. It is of interest to note that the labeled blast cells followed an identical pattern. This suggests a) that the labeling procedure did not interfere with the viability of the labeled cells; b) that the kinetic behavior of the unlabeled cells was not appreciably influenced by Methotrexate considering that the labeled cells were exposed to lower concentrations of Methotrexate than the unlabeled cells, due to the rapid plasma clearance of Methotrexate (1 6).

A quick and very pronounced decline in the blood blast cell count as observed here could have several reasons. One such possibility would be that a reduction in the number of actively proliferating marrow cells induces Q-cells to resume proliferation. Return of the predominantly quiescent blood blast cells to the marrow might be part of this process. It should be noted in this context that the environment outside of the hemopoietic organs appears little conducive to blast cell proliferation, at least mitoses in the blood are exceedingly rare (8) also when compared to the number of cells in DNA synthesis (10). It is possible, then, that the rapid and marked decrease in the blood blast cell concentration which may take place after single injections of cytostatic drugs, and which frequently seems out of proportion to the decrease in marrow cellularity does not only reflect leukemic cell kill but may also be due to a redistribution of cells between blood and marrow The only alternative would appear to be that drugs such as Methotrexate, which are supposed to inhibit actively proliferating cells, may also kill cells in the quiescent state.

2. *Return of blast cells from blood to marrow*

As seen from Fig. 1 and Table I, fair numbers of labeled cells were present in all bone marrow smears. In interpreting these figures it is obviously important to determine whether the labeled cells in the marrow could simply be the result of admixture of peripheral blood to the marrow samples. It is apparent that at most points of time the percentage of labeled cells was lower in the marrow than in the blood, however higher percentages in marrow than in blood were observed

at 26 and 96 hours after the autotransfusion. Moreover the figures corrected for upper limit *blood contamination with labeled blasts demonstrate* the presence of labeled cells in the marrow proper at no point could contamination of marrow aspirates with peripheral blood possibly account for the number of labeled blast cells observed in the marrow smears. This method of correction reflects and confirms that the cellularity in the marrow smears was considerably higher than in the corresponding blood smears. This was particularly conspicuous at the later points of time when the blood was very leukopenic. Also, the ³H-thymidine data (Table I) militate against significant blood contamination. In the bone marrow aspirated just prior to the study the thymidine labeling index was 10 times higher in the marrow than in the blood. In subsequent marrows the thymidine labeling index in the marrow doubled (as a result of Methotrexate (4)) and remained above the pretreatment level throughout the study

Another possible source of error would be labeling of marrow cells in vivo from free ³H cytidine contained in the autotransfused blood. This is unlikely because the mean grain counts of labeled blast cells were high and very similar in all corresponding blood and marrow samples (Table I). That some very slight labeling of cells in DNA synthesis may have taken place is considered later

In interpreting the percentages of labeled blast cells in the marrow it must be kept in mind that the cellularity was higher in the marrow than in the blood, particularly in the late phase of the study A quantitative interpretation of the data would require knowledge about the relevant pool sizes. The size of the marrow blast pool is not known, but probably was similar to that of the blood pool. From the blood blast count and the estimated blood volume the blood blast pool is calculated to be ∠6 × 10 ¹ From this and the percentage of labeled blood blasts the recovery of *autotransfused labeled cells would be close to* 100%. Since fair numbers of labeled blast cells were seen in the marrow even before the blood blast count had started to decrease, it is likely that the estimate of the patient's blood volume is too high and that the infused blast cells had rapidly been distributed between blood and marrow Although the fate of the labeled cells cannot be accounted for quantitatively it is concluded that, at least under the conditions of the study leukemic myeloblasts may return from the blood *to the bone marrow*

A rather surprising feature is the stability of the concentration of labeled cells in the blood during the first 16–20 hours. There is no explanation for this except to note that this period was the prelude to an extremely severe perturbation of steady state conditions.

Another interesting aspect is the relative stability of the ³H-cytidine label (Table I). At the time of H-cytidine labeling only 0 9% of the blood blasts were labeled with ³H-thymidine, and in this case, therefore, ³H-cytidine was almost exclusively a label of RNA.

3 *Do blood myeloblasts resume proliferation after return to the bone marrow*

Besides determining whether blasts would recirculate from blood to marrow it was also an aim of the study to obtain evidence whether such returning blast cells would divide in the marrow Methotrexate was administered to depopulate the proliferating fraction of the leukemic population Vincristine was given to arrest cells in mitosis a significant accumulation of labeled mitotic figures would have been rather direct evidence that quiescent cells (which are the bulk of the blood blasts) had entered into active proliferation The data do not allow any conclusions on this point. At the time of maximum vincristine effect about 13% of the mitotic figures were labeled, and in subsequent samples about of mitoses were labeled. However the grain counts of these labeled mitoses were much lower than in labeled interphase cells. It is not clear how these mitotic cells acquired their slight labeling It is unlikely that it represents in vivo uptake of free ³H-cytidine by proliferating blasts at the time of the labeled autotransfusion, in that case one would have expected higher degree of labeling of mitotic figures in *the early marrow samples* than in later samples, which is not borne out by the data. A more likely possibility is that the labeling was due to reutilization of ³H-cytidine from the in vitro labeled blast cells by proliferating blasts. Finally it must be considered that these lightly labeled mitotic figures may after all have represented Q-cells that had gone back into cell cycle, in that case it would be necessary to assume that the processes involved

Table I *Serum and urinary creatine and creatinine in eight patients with paralytic poliomyelitis*

Pat. no		Serum Creatine (mg%)	Serum Creatinine (mg%)	Serum Creatine/creatinine (ratio)	Urine Creatine (mg/24 h)	Urine Creatinine (mg/24 h)	Urine Creatine/creatinine (ratio)
1		2.3	0.48	4.8	390	165	2.36
2		1.8	0 13	13.8	335	177	1.89
3		1.3	0 20	6.5	348	334	1.04
4		2.3	0.46	5.0	610	675	0.90
5		3.1	0.24	13.0	712	333	2.14
6		1.8	0.16	11.3	643	397	1.62
7		1.6	0.11	14.5	394	271	1 46
8		1.8	0.23	7.8	465	239	1 95
Polio	$\bar{x}$	2.0	0.25	9 6	487	324	1.67
	S.D	0.52	0.12	3.7	138	152	0 48
	S.E.	0.18	0.05	1.3	48	54	0 17
		8	8	8	8	8	8
Normal subjects	$\bar{x}$	0.45	0.79	0.59	78	1 539	0.056
	S.D	0.14	0.13	0.18	44	340	0 039
	S.E.	0.016	0.015	0.021	14	144	0.013
		78	78	78	9	9	9
	p	$<0\ 001$	<0.001	<0.001	<0.001	$<0\ 001$	$<0\ 001$

excretion in all cases but one. This is a very infrequent finding.

The changes observed seem to be attributable to the inactivity of the musculature in paralytic poliomyelitis. The creatine formed in the kidneys, liver and pancreas cannot be utilized by the muscles and is therefore excreted in the urine. Consequently the formation of creatinine is very slight. Using radioactive isotopes, Roche et al. (7) and Benedict et al. (1) showed that in progressive muscular dystrophy the urinary creatine is not derived from the muscles, it represents recently synthesized *creatine, which has not parti*cipated in the muscular metabolism.

The clinical implication of these observations is that in paralytic poliomyelitis renal function cannot be evaluated on the basis of the serum creatinine concentration. Urea and clearance determinations have to be used instead.

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ACUTE RENAL INSUFFICIENCY DURING TREATMENT WITH AZATHIOPRINE

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Abstract. Fever muscular pain, gastrointestinal symptoms, oliguria and severe reduction of renal function developed in 49-year-old man, suffering from chronic glomerulonephritis, after 11 days of treatment with azathioprine, 3.4 mg/kg/day During later attempt at treatment with 1.1 mg/kg/day the same picture was reproduced after few hours. The kidney damage was probably part of hypersensitivity reaction to azathioprine. The patient later tolerated treatment with cyclophosphamide.

Renal side-effects due to azathioprine to our knowledge have hitherto not been reported. This paper describes case of acute renal insufficiency presumably caused by a hypersensitivity reaction to azathioprine.

CASE REPORT

The patient was 49-year-old man. In Dec. 1966 the patient had an operation elsewhere for arthrosis in the right hip Protein, leucocytes and erythrocytes were found in the urine and the serum creatinine was 1.0 mg%. The findings were ascribed to cystitis, and no treatment as instituted.

After that time the urine was often browny and the patient occasionally had pain in the loins and in the right hip There had never been oedema or sore throat.

On Feb. .., 1968, the patient was admitted to our department on account of pain in the left side of the thorax. After few days oedema appeared on the legs. X-ray showed a small exudate and an infiltration at the base of the left lung, which cleared up during the following eeks. The heart was enlarged, but the ECG was normal. The urine contained 8–13 g protein/24 hours and 30–50 erythrocytes, 10–15 leucocytes, 0–2 epithelial cells, several hyaline casts nd few granular casts per high power field. The serum creatinine concentration was 1.3 mg% and the creatinine clearance 55 ml/min.

Intravenous urography as normal. The haemoglobin was 14.7 g/100 ml and the hite cell count 9 100/μl with normal distribution.

The blood pressure was 170/95 mmHg Investigations for LE cells, antinuclear factor and rheumatoid factor were negative. Antistreptococcal titres ere normal.

Paper electrophoresis of the serum proteins showed the pattern of nephrosis (albumin 1.78 g/l, γ 0.69 g/l, α_2 1.34 g/l). Se-cholesterol 350 mg/100 ml.

A renal biopsy on Feb. 20 showed chronic glomerulonephritis of proliferative type with some epithelial crescents (Fig. 1).

During the following 4 months the serum creatinine rose to 1.5 mg% but the creatinine clearance was stationary at about 50–60 ml/min and urine protein xcretion was about 10 g/24 hours.

The first treatment ith azathioprine was instituted on June 14. The oral dose was 300 mg/day (3.4 mg/kg). During the first days of therapy the patient complained of nausea and sensations in the left hypochondrium. From June 25 the temperature rose, there was pain in the left hypochondrium and pain and stiffness in the neck, although no rigidity could be demonstrated (Fig 2). The urine volume decreased to about 350 ml/24 hours, serum creatinine rose to 7 mg/100 ml and the creatinine clearance fell to 9 ml/min.

After gradual reduction in dose (from June 26) treatment ith azathioprine was stopped on July 1 During the next 4 hours the temperature fell to normal. In the following days an exanthema resembling erythema multiforme appeared, and for more than week there was muscular pain in the legs.

At no time had there been hypotension or signs of dehydration. Haemoglobin values dropped from 12.6 g/100 ml (June 12) to 7.2 g/100 ml (July 2). There were no indications of haemolysis (normal reticulocyt count, elevated haptoglobin) White cell count was permanently about 6 000–7 000/μl with single low value of 3 800/μl (July 3) Differential count as practically normal except for slight increase in eosinophils (to 8) on tw occasions (July 1 and 12). Platelet count as normal or elevated. The leucocyte count in the urine sediment rose to max. 70–80 cells per high power field (June 29), but the urine as sterile on several examinations. Repeated blood cultures ere all sterile

A biopsy from the right gastrocnemius muscle on Aug. 30 showed cicatricial changes consistent ith toxic myopathia.



Fig. 1. Part of first kidney biopsy specimen showing a renal corpuscle with a marked proliferation of mesangial cells and increased lobulation of the vascular tuft. A hyaline thrombus and an adhesion is seen at the bottom. Silver-methenamine. Original magnification, × 400.

The patient's general condition deteriorated during the episode. Afterwards there was a pronounced tendency to retention of fluid. Creatinine clearance remained at values of 10–20 ml/min.

As the episode was presumed to be an exacerbation of the primary disease, which was supposed to respond to immunosuppressive therapy, *treatment with azathioprine was resumed* on Sept. 9, 1968, with lower doses (100 mg/day) (Fig. 2).

Four hours after the first dose the patient developed malaise, shivering, fever, nausea, vomiting, diarrhoea, headache and pain in the right side of the thorax and in the loins.

These symptoms were not recognized until the next day when a further dose had been given. About four hours after this dose the patient had the same symptoms, now more pronounced.

Treatment with azathioprine was immediately stopped. However, the patient developed acute renal insufficiency with oliguria, hyperkalaemia and increasing serum creatinine concentration. Creatinine clearance dropped to 0.7 ml/min. Culture of the urine was positive on Sept. 11

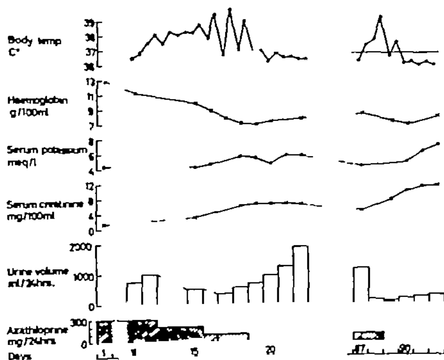


Fig. 2. Diagram showing urine volume, temperature, various biochemical data and dosage of azathioprine. The abscissa indicates the number of days passed since the start of the first period of treatment.

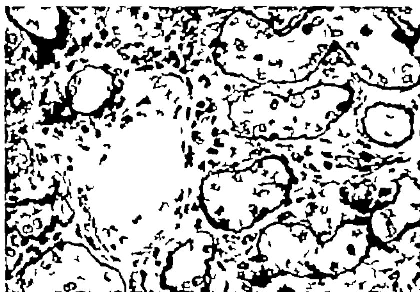


Fig 3 Second kidney biopsy specimen with section from the cortex showing loss of normal tubular structure: atrophy of the epithelium, local disappearance of the basement membrane, occurrence of hyalin casts, epithelial cell necroses and strongly varying appearance of the cells in single cross-section, often with vacuolisation. In the interstitium leucocyte infiltration is seen.

and 13 but the pattern was consistent with contamination, and new culture on Sept. 14 was negative, although no antibiotic was given. Repeated blood cultures were negative. There had been no hypotension or dehydration. There was slight fall in haemoglobin (8.8 to 7.3 g%). White cell count was normal but with slight shift to the left. No eosinophilia. Treatment with mannitol, 25% 30 ml 1 had no effect.

On Sept. 14 the patient was transferred to Nephrological Ward (Kommunehospitalet, Third Medical Department), where peritoneal dialysis was performed.

A new renal biopsy on Sept. 17 now showed the renal corpuscles to be dominated by intense epithelial capsular proliferation with formation of crescents. There were signs of acute and chronic tubular changes (Fig 3), and in the interstitial tissue there was considerable fibrosis and infiltration with leucocytes, particularly with many eosinophils.

After the dialysis the creatinine clearance improved to level of about 10 ml/min, and the patient was referred to Medical Department B, Bispebjerg Hospital.

The relentless course of progressing uraemia was not influenced by trial with cyclophosphamide (Endoxan®) 150 mg/day The patient died on Feb 7 1969

Autopsy showed large, pale kidneys (4 7 15 cm) with pronounced microscopic changes corresponding to chronic glomerulonephritis with numerous crescents. N signs of urinary tract infection were present. The heart was enlarged (600 g) and there as uraemic pericarditis.

DISCUSSION

Our patient suffered from chronic glomerulonephritis, verified by kidney biopsy On two occasions, both related to treatment with azathioprine, he developed an acute condition characterized by fever gastrointestinal symptoms, pain and oliguric renal insufficiency which was only partly reversible. The first episode was accompanied by a fall in haemoglobin and followed by a period of rash and myopathia.

As renal damage was, to us, an unknown complication of treatment with azathioprine, the oliguria was at first regarded as a nonspecific deterioration due to the chronic glomerulonephritis. However after the second episode it was clear that there might be a relationship between the azathioprine medication and the renal damage.

The toxic effects of azathioprine are well known and consist first and foremost of bone marrow depression and gastrointestinal irritation (3, 4 5). They are often dose-dependent. The second and most severe reaction in our patient occurred after a very small dose, and we therefore find it improbable that is was caused by a toxic effect of azathioprine on the kidney

A more reasonable explanation of the acute changes in renal function is that they were part of a hypersensitivity reaction to azathioprine. This concept is supported, although not proved, by the following facts.

1 Both reactions occurred during treatment with azathioprine, and no other provoking factor than this drug and the kidney disease itself could be demonstrated.

Thus there was no infection, shock, haemolysis or urinary tract obstruction. Nor was any drug

given which was not well tolerated on a later occasion.

2. A latency period of 11 days preceded the first reaction, while the second began a few hours after the administration of azathioprine.

3 Improvement, especially an immediate fall in temperature, followed the discontinuation of azathioprine.

It is peculiar that azathioprine, which is known to depress the antibody response, should be able to act as an antigen, but the possibility cannot be rejected. As the drug has a rather low molecular weight, it should operate as a hapten attached to a body protein. Whether the hapten is azathioprine itself or one of its degradation products (2) is an open question.

A reaction to azathioprine resembling the present, but without renal failure, was reported by Corley et al. (1). A patient with idiopathic thrombocytopenic purpura developed fever arthralgia and myalgia after two weeks of therapy The symptoms were reproducible upon re-institution of therapy and cleared rapidly on cessation of treatment.

Azathioprine is commonly used in combination with other immunosuppressive agents and the patients may be severely ill with symptoms from several organs. Especially in patients with diseases of the kidney or after renal transplantation it may be difficult to determine whether a sudden decrease in renal function reflects an increased activity in the primary kidney disease (or a rejection reaction respectively) or is a complication of the treatment. Although admittedly it must be rare, *it is important to look for the latter possibility*

ACKNOWLEDGEMENT

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DANGEROUS REDISTRIBUTION OF THALLIUM BY TREATMENT WITH SODIUM DIETHYLDITHIOCARBAMATE

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Abstract. In patients with thallium poisoning, treated with the chelating agent sodium diethyldithiocarbamate (dithiocarb), clinical deterioration and electroencephalographic disturbances have been observed coincident with the periods in which dithiocarb was given. Intravenous administration of dithiocarb to rats poisoned with thallium causes redistribution of thallium in the chelate form, with an increase of concentration in the brain. The chelate formed is rapidly decomposed again. These observations constitute a contraindication to the use of dithiocarb as an antidote in thallium poisoning.

Adverse reactions were seen in patients with thallium poisoning treated with sodium diethyldithiocarbamate (dithiocarb), which is not itself toxic (8). During intravenous infusion of dithiocarb loss of consciousness was noted, and when a continuous registration of the electroencephalogram was made, progressive deterioration was seen (Figs. 1, 2, 3). Although consciousness was regained a few hours after the end of the infusion, the electroencephalographic disturbances persisted for several weeks. Urinary thallium excretion rose substantially when dithiocarb was given, as did the thallium concentration in the blood. Some of these observations were reported previously (3). (EEGs recorded and interpreted by the Department of Electroneurology, Prof. Dr W. Storm van Leeuwen, University Hospital, Utrecht.)

Deterioration of cerebral function is not seen in the first few hours after thallium ingestion, when thallium levels in the blood are higher than later on in the course of the intoxication. In most cases of serious thallotoxicosis neurological symptoms are not encountered until after one week, and sometimes later. So not the thallium ion itself but the chelate was suspected to be the cause of the disturbances in cerebral function. These disturbances were thought to be related to a redistribution of thallium in the chelate form. Experiments with animals were made to test this hypothesis. In the course of the experiments the analogous thallium chelate of bis(hydroxyethyl)-dithiocarbamate was also studied.

MATERIAL AND METHODS

Thallium in tissues was determined according to Wolf and Lenstra (8). When thallium chelate was to be determined separately tissue samples of about 1 g were homogenized in 10 ml 0.01 M sodium hydroxide immediately after their removal from the sacrificed animals. The homogenate was extracted three times with 5 ml methyl-isobutyl-ketone. After separation the solvent was evaporated and thallium was determined in the residue and in the extracted homogenate. The former was considered to represent thallium chelate, the latter free and otherwise bound thallium.

In the redistribution experiment three groups of five female Wistar rats (approximately 150 g body weight) received 0.1 mM/kg thallium nitrate in glucose 5% intraperitoneally. After 24 hours the thallium had reached its characteristic distribution pattern (4). Then the animals were treated with either 10 ml/kg saline (control group), or 0.1 mM/kg sodium diethyldithiocarbamate (Na-DDC group), or 0.1 mM/kg sodium bis(hydroxyethyl)-dithiocarbamate (Na-BHDC group), both intravenously dissolved in saline. After another 4 hours the animals were sacrificed and thallium was determined in blood, brain, liver, kidneys, small and large intestine including their contents, and muscle. The quadriceps was isolated from hind limb and was used as sample of muscle tissue.

In the time course experiment five groups of four rats were treated with 0.1 mM/kg thallium nitrate intravenously. After 4 hours saline was administered intravenously to two control groups; 0.1 mM/kg sodium diethyldithiocarbamate in saline was administered intra-

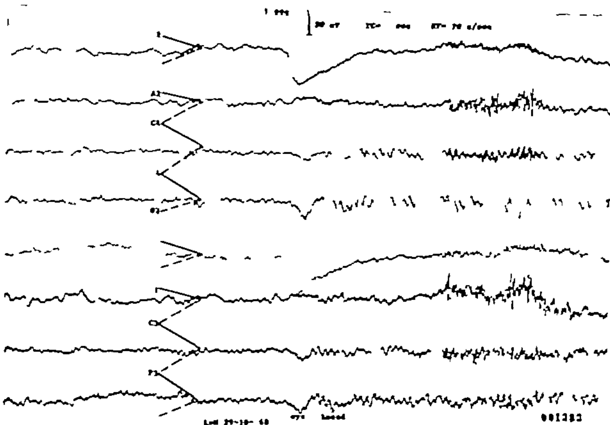


Fig 1 EEG recorded 29 10.68, two days before dithiocarb administration. At eye closure an alpha rhythm occurs at 9–10 c/sec, 30–40 μV (electrodes placed and indicated according to 10–20 system). Paper speed 3 cm/sec.

variously to the three other groups. At different times animals were sacrificed and the brains immediately excised, homogenized in 0.01 M NaOH, and subsequently extracted with methyl-isobutyl-ketone.

In the stability experiment thallium-diethyldithiocarbamate was incubated with a 40% rat liver homogenate in pH 7.4 phosphate saline buffer at 37°C. At different times homogenates were brought to pH 12 with sodium hydroxide and extracted with methyl-isobutyl-ketone.

Significance of differences was assessed by Wilcoxon's two sample test.

RESULTS

The results of the redistribution experiment are shown in Table I. No great differences are seen between the treated group and the control group as regards liver kidney and blood levels. However the thallium concentration in the brain in the treated groups is more than doubled ($p<0.01$). On the other hand, thallium concentration in muscle is slightly less in the treated groups compared to the control group.

In Fig. 4 the time course of the thallium concentration in rat brain after treatment with sodium diethyldithiocarbamate and after control treatment is demonstrated. In the latter case there is a slow rise to levels of about 7 μg/g. From other experiments (6) it is known that in untreated animals no concentration maximum occurs in the first 24 hours. Treatment with sodium diethyldithiocarbamate causes a sharp rise in brain thallium concentration, followed by a slow decrease. No thallium chelate has been detected. Fig. 5 shows the combined results of these experiments and of two previous studies (3 6).

In the stability experiment a half-life of thallium chelate of approximately 0.5 hour is found (Fig. 6).

DISCUSSION

Dithiocarb was given for the first time in the therapy of thallotoxicosis in 1963 by Bass (1). Sunderman (7), Moeschlin (5) and Gleason et al.

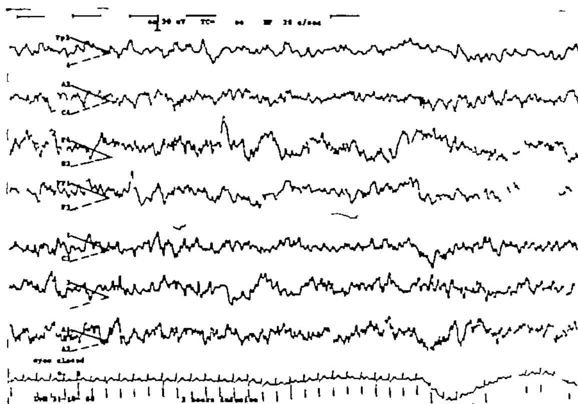


Fig. 2. EEG recorded 31.10.68 during 3 h infusion of dithiocarb. Alpha rhythm has disappeared; instead predominance of diffuse irregular activity at low frequencies, from 7 /sec down to 1 /sec, amplitudes 40–70 µV sometimes up to 100 µV. Arrangement of leads in this and following figures differs from that in Fig. 1 (paper speed in this and following figures 1.5 cm/sec).

(2) recommend the use of this chelating agent in cases of thallium poisoning. This recommendation is based on the rise of urinary thallium excretion observed after dithiocarb treatment of patients intoxicated with thallium. In our clinical experience this increase was confirmed, but severe side effects were observed.

Our experiments showed that thallium diethyldithiocarbamate is a lipophilic substance and has a rather short half-life in tissues. In the rat the toxicity of thallium as chelate is of the same order as that of thallium as nitrate (6). Intravenous administration of dithiocarb to rats, previously treated with thallium nitrate, doubled the brain thallium concentration compared with the untreated control group (Table I). An analogous experiment was made with sodium bis(hydroxyethyl)-dithiocarbamate. It was expected that the presence of two hydroxyl groups in the molecule would prevent the unfavourable change in the brain thallium concentration. This turned out not to be so.

On the basis of our findings we suppose that the following sequence of events occurs. The dithiocarbamate derivative chelates the thallium ion in the extracellular fluid. The extracellular free thallium concentration decreases, and hence intracellular unbound thallium is transported outward. The blood level of thallium (ionic and chelated) now rises, which may explain the increase of the urinary thallium excretion. The chelate circulates through the body and diffuses into the cells, meanwhile decomposing into thallium ion and dithiocarbamate metabolites. The thallium ion set free extracellularly is transported into the cells again as the dithiocarbamate concentration in the extracellular fluid declines. A new thallium distribution pattern now emerges, determined by the

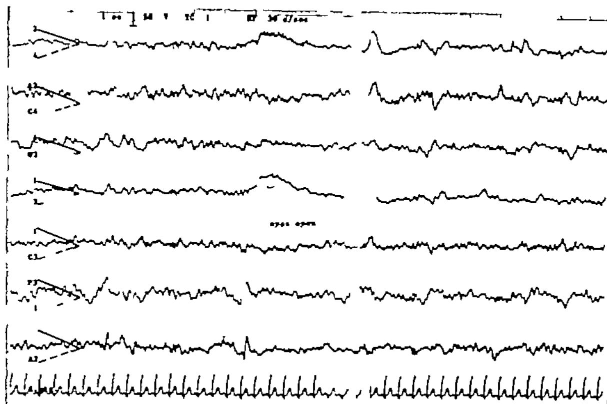


Fig. 3 EEG recorded 13.11.68 shows considerable decrease of abnormality: most of the activity at low frequencies has disappeared. The alpha rhythm has not yet attained the shape shown in Fig. 1. Thus the EEG is still diffusely abnormal.

distribution properties of the chelate and by the degree of vascularisation of the different tissue types. In Fig. 7 a scheme of the processes involved is given.

As the thallium chelate of dithiocarb is a lipophilic substance, and as the coma induced by dithiocarb therapy is only of short duration, we presume that the short living chelate is responsible for the coma, whereas the long lasting disturbances of the electroencephalogram are caused by the increased brain thallium concentration.

The explanation given applies primarily to

Table I. *Influence of administration of sodium diethyldithiocarbamate (Na–DDC) and sodium bis(hydroxyethyl)-dithiocarbamate (Na–BHDC) on thallium distribution in the rat*

Tissue concentrations of elementary thallium in μg/g wet weight ± S.D

Tissue	Control	Treated with Na–DDC	Treated/Control	Treated with Na–BHDC	Treated/Control
Blood	2.1 ± 1.4	1.8 ± 0.3	0.86	1.6 ± 0.4	0.76
Brain	6.6 ± 0.4	14.1 ± 0.8*	2.14	14.4 ± 4.2*	2.18
Liver	11.4 ± 1.7	12.9 ± 1.1	1.13	14.0 ± 1.7	1.23
Kidneys	83.9 ± 10.2	95.5 ± 9.6	1.14	84.1 ± 10.5	1.00
Intestine and contents	22.3 ± 2.5	24.8 ± 2.8	1.11	28.8 ± 2.8*	1.29
Muscle	22.2 ± 4.7	16.3 ± 1.5*	0.73	15.2 ± 2.4*	0.68

* Significantly differing from control group, $p < 0.05$.

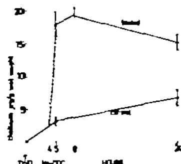


Fig. 4 Time course of thallium concentration in rat brain after consecutive intravenous administration of thallium nitrate and sodium diethyldithiocarbamate (interval 4 h).

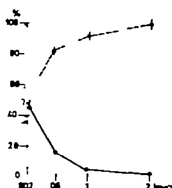


Fig. 6. Stability of thallium diethyldithiocarbamate in liver homogenate. ———— free thallium; ●——● chelated thallium.

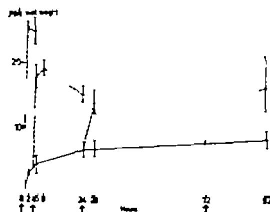


Fig. 5. Survey of time course of brain thallium concentration in the rat after parenteral administration of 0.1 mM/kg body weight, and deviations from the control group at various times after treatment with sodium diethyldithiocarbamate (↑). ▲ ▲ treated, ●——● control.

intravenous therapy with dithiocarb. As the same observations were made in the clinic after oral administration of dithiocarb this explanation may also be valid here. Dithiocarb therapy of thallotoxicosis thus appears to have some serious drawbacks: the chelate produced is lipophilic and will readily pass the blood-brain barrier. For the same reason it will be excreted slowly. Moreover it is decomposed rapidly in the body. As death after thallium poisoning is primarily caused by the neurological complications, the use of dithiocarb as an antidote in thallotoxicosis is contraindicated.

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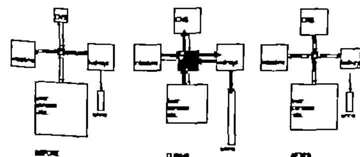


Fig. 7 Thallium distribution as influenced by administration of sodium diethyldithiocarbamate ▩, blood + extracellular fluid.

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Acta med. scand. Vol. 189 pp. 155–159 1971

PROLONGED AMBULATORY OXYGEN THERAPY IN PULMONARY HYPERTENSION OF VARIOUS ETIOLOGY

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Abstract. The effect of long-term (4 to 7 weeks) oxygen administration on the pulmonary arterial pressure has been studied in four patients with pulmonary hypertension of varying etiology. Two patients had congenital heart lesions; one had ventricular septal defect and the other Lutembacher syndrome and endocardial cushion defect. A third patient had pulmonary hypertension, history of Raynaud symptoms and heavy consumption of appetite-reducing agents. The fourth patient had pulmonary hypertension in combination with Mb Osler, as had her sister and probably other members of her family. None of these patients responded to chronic oxygen administration (2 to 10 l/min by nasal catheter) with fall in pulmonary arterial pressures, although there was rise in PaO_2 in all cases. Neither was any *fall in* pulmonary pressure seen when these patients were allowed to breathe pure oxygen for 10 to 25 min. This negative result is in contrast to other reports on long-term oxygen administration in patients with chronic bronchitis and emphysema. The effect of pure oxygen breathing on the pulmonary arterial pressure is suggested as a screening test for long-term oxygen administration to patients with pulmonary hypertension: if no acute effect of oxygen is seen on the pulmonary arterial pressure, there will probably be no chronic effect.

Encouraging reports have appeared recently on the beneficial effect of prolonged oxygen administration to patients with chronic airway obstruction (1, 3, 11): the patients felt better and the pulmonary artery pressure decreased. von Euler and Liljestrand (4) showed in 1946 that pulmonary vasoconstriction occurs in response to reduced oxygen tension. A number of studies have shown that the pulmonary hypertension of high altitude is reversible by descent to lower altitudes (5, 10). We therefore thought it worthwhile studying the effect of prolonged oxygen administration to patients with pulmonary hypertension of other etiologies.

MATERIAL AND METHODS

Four patients were studied. Care was taken to explain in detail to the patients the purpose of the study and that there was no guarantee of success. All patients but one (case 2) had undergone previous cardiac catheterizations and were thus familiar with the procedure. After few days hospital acclimatization, right heart catheterization was performed, after which oxygen was administered continuously for at least four weeks.

Technical method

Arterial pressure was measured through polyethylene catheter inserted into brachial artery by the Seldinger technique under local anesthesia or through an Ödman catheter introduced into the aorta through the femoral artery. A right heart catheterization was performed with Cournand catheter.

Intravascular pressure recordings were obtained with four-channel direct-writing ink jet electrocardiograph (Mingograph 42 B, Elema-Schönander, Stockholm). Mean pressures were obtained by electrical integration over period including at least two respiratory cycles. The patients were recumbent, and the zero level for the strain gauges during the investigation was the mid-thoracic line measured at the sternal insertion of the fourth rib. Cardiac output was determined in duplicate by dye dilution technique, 3 ml of 5% sodium bromsulphalein solution (13) being rapidly injected into the pulmonary artery or by the Fick method, oxygen consumption being collected during 5-min period. Hematocrit was determined in duplicate on arterial blood drawn into an Ellermann tube. After thorough mixing, 75 mm heparinized microhematocrit tube was filled and centrifuged at 8 000 r.p.m. for at least 5 min.

Arterial pH was measured by conventional technique. The arterial oxygen tension (PaO_2) was measured with modified Clark electrode (2), and arterial carbon dioxide tension ($PaCO_2$) according to Severinghaus and Bradley (12). Standard bicarbonate was determined according to Jørgensen and Astrup (7).

Clinical procedure

The patients were admitted to hospital few days before the start of the study. During this initial period, and

Table I. *Random measurements of blood gas tensions and acid base state before (B) and during continuous oxygen (Roman figures, week of oxygen administration)*

Case	O_2 administration (week)	O_2 administration (l/min)	PaO_2 (mmHg)	$PaCO_2$ (mmHg)	Arterial pH	Standard bicarbonate (mEq/l)
1	B	0	26	51	7.41	30
	I	6	51	63	7.3[illegible]	33
	II	6	40	57	7.44	35
2	B	0	72	31	7.54	26
	VI	2	103	40	7.51	30
3	B	0	68	32	7.48	23
	I	5	122	42	7.42	26
	III	5	143	36	7.49	26
4	B	0	64	36	7.39	21
	I	4	86	38	7.48	27
	III	10	140	40	7.45	26

throughout the stay in hospital, treatment, including digitalis and diuretic, was continued. Oxygen was administered at 2 to 10 l/min through nasal catheter. Random measurements of arterial blood gas tension (Table I) showed that this treatment raised the PaO_2 to normal or supernormal values except in case 1 though even in this patient rise was noted.

A cardiac catheterization with measurement of intracardiac pressures and blood flow was performed just before the start of oxygen administration, except in case 2, in whom it was performed five months before oxygen. In each case the procedure was repeated after 4 to 7 weeks of oxygen administration. Oxygen was given until one half to two hours before the second catheterization.

CASE REPORTS

Case 1

Female, born in 1923. Kyphoscoliosis and dextrocardia known since her childhood. In 1952 she became cyanotic on exertion, and later dyspnea and tiredness appeared. In 1966 iterated hemoptyses started. Since then her condition has deteriorated continuously. Cardiac examination revealed a pulmonary hypertension with mean pulmonary pressure of 82 mmHg and mixed shunt. She had typical "scimitar" syndrome (9) with an anomalous pulmonary vein with blood from the major part of the right lung emptying into the inferior vena cava below the diaphragm, furthermore an endocardial cushion defect, dextrocardia, displacement of the aorta to the right and backwards with slight coarctation of the aorta. Her vital capacity was 0.87 l, residual volume 1.38 l, forced expiratory volume (1 sec) 0.49 l, and lung clearance index 14.9. Her coagulation findings were normal apart from highly increased adhesiveness of the thrombocytes. Her cardiac decompensation was treated with digitalis, diuretics and aldosterone antagonists. No subjective improvement was noticed after oxygen administration for five weeks.

Summary

A 44-year-old woman with dextrocardia, kyphoscoliosis, scimitar syndrome, endocardial cushion defect, aortic anomalies and pulmonary hypertension.

Case 2

Female, born in 1919. Jewish woman who came from Poland to Sweden in 1945. From 1958 to 1968 she used appetite-depressing agents of various kinds. In Jan. 1968 an arterial hypertension of 185/115 mmHg was found and she was given diuretics. She complained of symptoms of Raynaud type. Thoracic pain developed, as did dyspnea, tiredness and dependent edema. Cardiac examination in Aug. 1968 revealed loud fixed split second sound, an enlarged heart with total volume of 1 050 ml, corresponding to 700 ml/sq.m BSA, right ventricular hypertrophy in the ECG, pulmonary hypertension with mean pulmonary pressure of 56 mmHg, and normal PCV pressure, 2 mmHg. Angiography showed an enlargement of the main pulmonary arteries without stenoses, and narrow peripheral pulmonary arteries, finding consistent with primary pulmonary hypertension. Intravenous pyelography revealed cysts of the kidneys. Her creatinine clearance was normal. Her peripheral circulation was impaired, with faint radial pulses; venous occlusion plethysmography showed reduction of the leg flow. Her coagulation findings were normal apart from an increased adhesiveness of the thrombocytes. Spirometric lung function values were normal. She was given oxygen for seven weeks in the hospital, and afterwards intermittently at home. The second catheterization was performed in Feb. 1969 after seven weeks of oxygen administration. She was improved subjectively but not objectively. Oxygen treatment continued intermittently until her death in Aug. 1969. Autopsy showed right ventricular hypertrophy and no signs of pulmonary emboli. In the pulmonary artery and its larger and medium-sized branches there was very prominent atherosclerosis. Microscopy of peripheral lung sections revealed areas of

Table II. *Physical characteristics, duration of continuous oxygen administration and hematological data*

I = control before oxygen administration was started. II = end of long-term oxygen.

Case		Age (y.)	Weight (kg) I	Weight (kg) II	Hemoglobin (g/100 ml) I	Hemoglobin (g/100 ml) II	Red blood corpuscles (/mm³) I	Red blood corpuscles (/mm³) II	Duration of oxygen administration (weeks)
1	♀	46	52.5	55.3	12.5	13.9	(4.4)	4.4	4
2	♀	50	64.2	60.0	14.1	13.4	4.6	5.0	7
3	♀	48	53.3	53.0	11.6	10.6	3.8	—	4
4	♂	35	55.5	57.5	15.0	15.4	4.7	—	5

pronounced hyperplasia of the intima in the smallest artery ramifications. A certain hyalinization in the media of some small arteries was also seen. Both kidneys had large numbers of retention cysts, but fairly abundant microscopically normal renal tissue remained. A detailed history of this patient, including list of the appetite-depressing agents, was published by Malmquist et al. (8).

Summary

A 50-year-old woman with heavy consumption of appetite-reducing agents, Raynaud phenomenon, cysts of the kidneys and pulmonary hypertension of "primary" type.

Case 3

Female, born in 1921. A sister had Mb Osler and pulmonary hypertension. The patient early complained of dyspnea and tiredness. Typical Osler changes later resulting in nose bleedings and melena were first noticed in 1950. In 1955 diagnosis of pulmonary hypertension. Her mean pulmonary artery pressure was 38 mmHg on this occasion, and her PCV was normal, 8 mmHg. Her dyspnea, tiredness and repeated bouts of anemia secondary to her Osler disease progressed, and in 1965 her mean pulmonary pressure was 47 mmHg, and in 1969 60 mmHg. Her coagulation findings were normal, as was her lung function. Her heart volume showed steady increase from 880/570 ml/sq m BSA in 1955 to 1 100/710 in 1969. The electrocardiographic signs of right ventricular hypertrophy also progressed. An angiocardiography showed enlargement of the right ventricle and pulmonary artery. The peripheral pulmonary arteries were narrow and there were no signs of shunt. She was hospitalized in 1969 for four weeks of continuous oxygen treatment.

Summary

A 48-year-old woman with familial occurrence of Mb Osler and pulmonary hypertension.

Case 4

Male, born in 1934. A congenital heart lesion was diagnosed just after birth. In 1950 ventricular septal defect with pulmonary hypertension as found with pulmonary pressure of 80/29 mmHg; the corresponding value in 1961 was 107/47 mean 74 mmHg, and no decrease was obtained after Priscol administration. Pronounced symptoms began in 1962, and his condition has deteriorated with increasing dyspnea, angina pectoris and hemoptysis. He has an enlarged heart with volume of 1 190/700 ml/sq m BSA, and there are electrocardiographic signs of right ventricular hypertrophy. A grade 5 systolic murmur is heard in the left fourth interspace, as is loud second sound and diastolic decrescendo murmur over the sternum. He was hospitalized in 1969 for five weeks of continuous oxygen administration, which he afterwards continued intermittently at home.

Summary

A 35-year-old man with ventricular septal defect and pulmonary hypertension, first diagnosed in 1950.

RESULTS

The results of blood gas tensions and acid base state are given in Table I. Values for body weight, hemoglobin, red blood corpuscles and duration of oxygen administration are given in Table II, and hemodynamic values in Table III.

Period of oxygen therapy. PaO_2 was below normal in all patients when breathing air. Oxygen administration resulted in an increase in PaO_2 in all patients including case 1 who had mixed shunt, although the rise in this patient was less pronounced than in the others. As will be seen from Table I, PaO_2 was dependent on the percentage of oxygen in the inspired air; this is still more obvious from Table IV. $PaCO_2$ rose in all patients during oxygen administration, but only moderately even in case 1 who also had an elevated $PaCO_2$ value when breathing air, and there were no signs of carbon dioxide narcosis. No consistent changes appeared in standard bicarbonate or arterial pH. The weight remained mainly unchanged, as did the hemoglobin and erythrocyte values (Table II). No beneficial effect

Table III. *Hemodynamic data referring to long-term oxygen administration*

I = control before oxygen administration was started. II = end of long-term oxygen.

Case	Cardiac output (l/min)		Stroke volume (ml)		Heart rate (/min)		Blood pressure (mmHg): Pulmonary artery: Systolic		Diastolic		Mean		Brachial artery: Systolic		Diastolic		Mean	
	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II
1	3.7	—	37	—	101	108	108	120	67	69	87	84	124	130	73	80	91	100
2	3.2	2.7	36	27	88	98	95	97	38	47	56	65	146	122	110	97	120	100
3	6.5	6.4	81	83	80	78	94[a]	95[a]	—	—	—	—	96	95	52	56	69	70
4	8.6	11.9	121	195	63	61	121	108	52	51	81	72	120	110	72	64	90	80

[a] Right ventricular systolic values.

of long-term oxygen administration on the pulmonary artery pressure was observed (Table III). The slight decrease in case 4 is not significant when considering the changes occurring spontaneously and it was not accompanied by any subjective improvement.

Effects of pure oxygen breathing are shown in Table IV. After a resting period of at least 20 min, when the various hemodynamic data were obtained, the patients were allowed to breathe pure oxygen for 10 to 25 min, pressures were recorded, and flows were determined at the end of this period. The pressure values were recorded at intervals of two to four min and were found to fluctuate: not infrequently the systolic values rose 10 mmHg, but they decreased again later though usually not below the control value before pure oxygen breathing. Pressures were recorded in two patients after pure oxygen breathing had been followed by air breathing: in one patient no pressure changes occurred, in the other a moderate rise, 7–13 mmHg, of the mean pulmonary artery pressure.

DISCUSSION

The recent studies reporting a decrease in pulmonary arterial pressure after long-term oxygen administration to patients with chronic bronchitis and emphysema encouraged us to investigate the effect of long-term oxygen in other types of patients with pulmonary hypertension, not caused by a chronic bronchitis. The effect of acute administration of oxygen has been used in patients

Table IV. *Hemodynamic data referring to pure oxygen breathing*

B = before pure oxygen breathing. A = after 10 to 25 min pure oxygen breathing. I refers t catheterization before long-term oxygen administration; II to catheterization t the end of long-term oxygen administration.

Case		Cardiac output (l/min)		Heart rate (/min)		Mean blood pressure in mmHg: Pulmonary artery		Brachial artery		PaO_2 (mmHg)	
		B	A	B	A	B	A	B	A	B	A
1	I	3.7	5.4	101	100	87	90	91	90	30	49
	II	—	5.0	108	110	84	86	100	103	26	47
2	I	3.2	—	88	85	56	55	120	—	77	573
	II	2.7	2.2	98	98	65	61	100	105	61	—
3	I	6.5	6.0	80	71	92[a]	92[a]	65	65	69	640
	II	6.4	5.6	78	82	95[a]	98[a]	70	67	59	556
4	I	8.6	8.1	63	67	81	90	90	100	64	452
	II	11.9	4.5	61	61	72	74	80	82	56	535

[a] Right ventricular systolic values.

with pulmonary hypertension secondary to a congenital heart lesion with left-to-right shunt as indicator of surgery: a decrease after oxygen indicates a more favorable surgical prognosis than if the pressure remains unchanged. To this end we selected patients with pulmonary hypertension of varying etiology. One patient had "primary" pulmonary hypertension in connection with Mb Osler, another showed Raynaud symptoms and presented a heavy consumption of appetite-depressing agents which probably tend to elevate the pressure in the pulmonary arteries (6). The other two had congenital heart lesions with mixed shunts, one had a ventricular septal defect, and the other a scimitar syndrome with an endocardial cushion defect. All patients were in a bad condition, and three of them had been followed with repeated catheterizations indicating a slow but constant deterioration. Although one patient felt better during oxygen administration, none of the patients had objective hemodynamic findings indicating an improvement. That patient 2's impression of an amelioration was false, was confirmed by her death, half a year after the continuous oxygen administration had been stopped. Our negative results contradict the optimistic report by Petty and Finigan (11), that "It is likely that continuous oxygen therapy will be of therapeutic value for all disabled hypoxemic subjects whatever the underlying disease may be".

The reason for the disappointing result in these four patients is probably the different etiology. Abraham et al. (1) point out that there are characteristic histologic changes in the pulmonary arterioles in patients with chronic bronchitis and emphysema and evidence of pulmonary hypertension and right ventricular hypertrophy. One of these changes is a circular layer of smooth muscle in the media of the pulmonary arterioles, with little or no hyperplasia of the circular smooth muscle of the small muscular pulmonary arteries. Both classes of vessel also have layers of longitudinal smooth muscle internal to the internal elastic lamina, but do not have the internal fibrosis characteristic of other disorders associated with pulmonary hypertension. This difference could be of importance.

There are many etiologies of pulmonary hypertension, and our four patients certainly do not cover them all. It would be of value to have a screening test indicating whether it is worthwhile continuing long-term administration of oxygen. It is possible for these patients to be ambulatory and one of our patients had a portable oxygen equipment which he used at home. Abraham et al. (1) reported a fall in average pulmonary arterial mean pressure on acute administration of oxygen. We did not observe such a fall in our patients. It is therefore possible that this might be a screening test: patients who respond with a fall in pulmonary artery pressure on acute oxygen administration may benefit from long-term treatment.

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Table I *Influence of the age of patients on the early and late results following electrical conversion of atrial fibrillation (AF)*

Age group (y)	No of pats.	Primary and early failures (%)	Reversion to AF in 3 mo. (%)	Still in SR after 3 mo. (%)	6–12 mo. (%)
34–50	32	22	28	16	34
51–65	82	35	37	7	21
66–76	38	37	37	2	24

RESULTS

The influence of the age of patients, the aetiology of the underlying heart disease, the heart volume and duration of atrial fibrillation were studied in order to discover the relationship of these factors to the persistence of sinus rhythm after a successful electrical conversion. The effect of prophylactic quinidine therapy before and after the conversion was evaluated.

The results are seen in Tables I–VI. Cases in which conversion of atrial fibrillation to sinus rhythm was never achieved are called primary failures and those which, after a successful conversion, reverted to atrial fibrillation during the next four days while in hospital are called early failures.

The influence of the age of patients on the early and late results are seen in Table I. In the age group of 50 years and younger more patients, 50% retained the sinus rhythm for three months or more than did those in the older age groups. In the middle age group (51–65 years) and the oldest group (66–76 years) the long-term results were almost equal. The primary and early failures were fewest in the youngest age group.

The effect of the aetiology of the underlying heart disease on the results is presented in Table II. The numbers of patients in the different aetiology groups are very uneven, making the comparison difficult. It can be seen, however that the number of primary and early failures in treated hyperthyroidism and in lone fibrillation (atrial fibrillation without known primary heart disease) is surprisingly high. There were two cases in the group of hyperthyroidism which were thought not to be completely euthyroid during the defibrillation. After a few months of treatment and a careful evaluation of the patients, new

Table II *The aetiology of the underlying heart disease and the duration of sinus rhythm after successful conversion*

	No of pats.	Primary and early failures	Reverted to AF in 3 mo	Still in SR after 3 mo	6–12 mo.
Mitral incompetence or stenosis	38	9 24%	15 39%	4 11%	10 26%
Aortic insufficiency or stenosis	3	0	2	0	1
Combined rheumatic heart disease	5	2	1	0	2
Coronary heart disease	37	11 30%	16 43%	2 5%	8 22%
Hypertension	20	8 40%	5 25%	1 5%	6 30%
Treated hyperthyroidism	17	9 53%	2 12%	1 6%	5 29%
Myodegeneration	23	8 35%	9 39%	3 13%	3 13%
Lone AF	9	4 44%	2 22%	1 11%	2 22%
Total	152	51	52	12	37

Table III. *The effect of heart volume on early and late results*

Relative heart vol. (ml/m²)		No. of pats.	Primary and early failures (%)	Reversion to AF in 3 mo. (%)	Still in SR after 3 mo (%)	Still in SR after 6–12 mo. (%)
500	♂	13	31	23	0	46
–450	♀					
500–749	♂	85	30	39	7	24
450–749	♀					
751		54	37	31	11	20
Total	no.	152	50	53	49	
	%	100	33	35	32	

conversion attempts were made without success. Despite this, the persistence of sinus rhythm was much the same in the different aetiology groups, 26 to 40% of patients being still in sinus rhythm 3 to 12 months after the electrical conversion.

The heart volume had no significant effect on the primary and early failures, but the persistence of sinus rhythm was clearly better in patients with hearts of normal size than in patients with moderately or greatly enlarged hearts (Table III).

If the duration of atrial fibrillation before the countershock was less than two years, the number of primary and early failures was smaller about 30% than in the group with duration of atrial fibrillation for more than 24 months, 44%. Similarly the long-term results at 6 to 12 months were better in those patients who had had atrial fibrillation less than two years. The results were not better among the patients in whom atrial fibrillation had lasted less than 6 months compared with the group of atrial fibrillation for 6 to 24 months.

Quinidine therapy

It can be seen in Table V that quinidine prior to shock did not have a favourable effect on the conversion results. In fact, the number of primary failures was greater in the pretreatment group than in the group without pretreatment with quinidine ($p < 0.01$). There were less early reversions in the patients who were not given quinidine after the defibrillation shock than in those treated with quinidine ($p < 0.01$). The fact that the second half of the D.C. defibrillation series was more carefully selected than the first may to some extent, influence the results in favour of the no-quinidine group. There were four late deaths in the material, all reported earlier. Of these, one sudden death occurred 12 hours after successful cardioversion. The patient was otherwise in reasonably good condition, and the death was perhaps caused by an arrhythmia due to quinidine toxicity. Immediately after the electric shock there were significantly more atrial and ventricular ectopic beats, ST-segment and T wave changes and other minor ECG abnormalities in the pretreatment group (72%) than in the other groups (39%). In one case short episodes of subsequent ventricular ectopic beats resembling ventricular fibrillation were seen in the quinidine group on the day after the defibrillation. Ventricular tachycardia was not seen at all.

The effect of quinidine on the long-term results can be seen in Table VI. There are no significant differences between the groups given quinidine

Table IV. *Duration of atrial fibrillation before and persistence of sinus rhythm after the electrical conversion*

Duration of AF (mo.)	No. of pats.	Primary and early failures (%)	Reversion to AF in 3 mo (%)	Still in SR after 3 mo. (%)	Still in SR after 6–12 mo. (%)
< 6	61	29	38	8	25
6–24	45	31	29	9	31
> 24	46	44	33	6	17

Table V *DC shock treatment of atrial fibrillation (AF). The effect of quinidine on primary failures and early (during the first 4 days) reversions to AF*

Drug therapy	No. of pats.	Primary failures No.	Primary failures %	Early reversions No.	Early reversions %
Pretreated with quinidine	76	17	22	29	26
Quinidine afterwards only	55			18	33
No quinidine	33	4	5	4	12
Total	164	21	13	51	32

and those that were not, but there may be some trend towards better results in the quinidine group, 6% more patients of the treated group retaining the sinus rhythm at 6 and 1_ months. Group III contained 11 patients who did not tolerate quinidine and were therefore transferred to this group. Thirty-three patients of groups I and II complained of gastrointestinal disorders connected with the administration of quinidine. There were several patients who did not fully cooperate in

Table VI. *Electrical conversion of atrial fibrillation. number of patients in sinus rhythm after successful conversion*

Group I treated with quinidine
Group II stopped quinidine after 3–6 months
Group III no quinidine

Follow-up period	Group I SR	Group II SR	Group II AF	Group III SR
Start	114			29
4 d.	81/114			25/29
1 mo.	53/107			14/24
3 mo.	43/107	6		11/24
6 mo.	31/105	14	3	7/24
12 mo.	9 80	5	12	2/22

Per cent of patients in sinus rhythm

Follow-up period	Group I+II	Group III	Total
Start	100	100	100
4 d.	71	86	74
1 mo.	50	58	51
3 mo.	40	46	41
6 mo.	35	29	34
12 mo.	14	8	13.4

taking quinidine, the true mean dose was about 0.8 g a day in the treated groups. Unfortunately no determinations of the plasma concentration of quinidine were available.

To summarize, one death could probably be attributed to the quinidine therapy and 44 patients showed some kind of intolerance to quinidine. Moreover there were more immediate post-conversional arrhythmias and other ECG changes in the quinidine-treated patients.

DISCUSSION

The value of DC defibrillation in terminating cardiac arrhythmias is obvious: the method is simple, *safe, effective and time-saving, as reported by* several authors. In the event of atrial fibrillation, sinus rhythm is achieved in 70 to 96% (6). In the present series 87% of conversion attempts resulted in sinus rhythm, this figure being near the mean number of reported results. During the next four days, when the patients were under observation in the hospital ward, as many as one third reverted to atrial fibrillation. After 6 months one third, and after 12 months 13% of cases with successful conversion were still in sinus rhythm. Our long-term results are even worse than those of most other authors, but they reflect the real problem in the treatment of atrial fibrillation: how to keep the patients in sinus rhythm after conversion? In the light of our results it seems likely that some selection of patients may improve the results.

Thus some advantage is achieved if the duration of atrial fibrillation has been less than two years, if the aetiology of the underlying heart disease is hypertension or treated hyperthyroidism, if the heart volume is within normal limits, and if the patient with atrial fibrillation is younger than 50 years of age. There are somewhat different opinions in the literature as to the effect of the above mentioned factors on the long-term results (1 3 9 1_, 16 17 18, 25). Most authors agree, however that the duration of atrial fibrillation is of importance when predicting the persistence of sinus rhythm after defibrillation, the critical limit of duration being from 2 years to 5 or some" years (1 3, 1_, _4).

It is possible that most differences in the results can be attributed to the selection of patients, composition of the series, and to differ

ences in the classification and evaluation of the results. It seems quite reasonable to accept that the time factor and the severity of underlying heart disease do have an influence on the results. This influence is, however, of only a limited, relative importance, as even in selected materials the one-year success is at the most 50% but in most reports less (49–14%). Thus the need for a safe and non-toxic antiarrhythmic drug for maintaining sinus rhythm is obvious.

Our experience with quinidine is disappointing: there were more primary failures in the group pretreated with quinidine than in the no-quinidine groups, and more early reversions in the quinidine-treated groups than in controls (Table V). Furthermore, the antiarrhythmic effect of quinidine in maintaining sinus rhythm was weak if any when groups treated with and without quinidine were compared (Table VI).

Korsgren et al. (12) and Rossi and Lown (21) recommend the use of quinidine prior to the shock, while others, e.g. Oram and Davies (17), Radford and Evans (18), Aberg (1) and Aberg and Cullhed (2) have completely abandoned quinidine administration prior to and after the electric shock because of quinidine syncope, sudden death, gastrointestinal intolerance and other side-effects.

Bjerkelund and Orning (3) reported eight cases of ventricular fibrillation and five of sudden death among 230 patients on quinidine therapy. When a more individual quinidine dosage, governed by the serum level, was given, no new attacks of quinidine syncope were observed in the last 117 patients treated (3). In Cramér series (5) thrombocytopenia was found in 26 out of 77 follow-up patients, and leucopenia and urticaria in one case, 61 patients out of 148 had gastrointestinal side-effects, and four out of 272 died a sudden death during sinus rhythm while on maintenance therapy with quinidine.

As we, too, in the present material, had one sudden death probably due to quinidine, frequent cardiac arrhythmias at conversion, ST and T-wave changes in post-conversion electrocardiograms (24), and several cases with gastrointestinal side-effects, we believe that quinidine is too hazardous a drug to be used in effective antiarrhythmic concentrations sufficient to prevent relapse (3, 12).

To conclude, a patient under fifty who has normal-sized heart and atrial fibrillation of only short duration (less than 2 years), has the best chance of maintaining sinus rhythm after conversion.

Unfortunately the patients who most need the increase in cardiac output achievable by conversion of atrial fibrillation into sinus rhythm are the least likely to hold sinus rhythm. It is, therefore, obvious that a more effective and safer drug than quinidine is needed for maintaining an achieved sinus rhythm after electrical conversion of atrial fibrillation.

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MEDICAL ASPECTS IN THE TREATMENT OF FEMORAL NECK FRACTURE

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Abstract. The aim of the study was to discover the diseases associated with fracture of the femoral neck treated in a surgical-orthopaedic hospital and, by systematic study to find the complications of the conservative, surgical and anaesthesiological methods of therapy. The material consisted of 166 patients, 135 of whom were females. The mean age was 71.9 years. In 20 cases the accident had occurred in other hospitals or in nursing homes. The mean duration of hospital treatment was 32 days. Medical examination, laboratory studies, ECG and chest X-ray were done in all cases immediately after admission and 7–10 days later or after operation. Additional diseases were found in 145 cases, the most important of which were heart failure in 72, an old myocardial infarction in 27, hypertension in 24, aortic stenosis in 21, diabetes mellitus in 16, and rheumatoid arthritis in 13. Metabolic alkalosis was found in 46. Respiratory function was measured in all cases. All patients over 60 were digitalized, and anticoagulants were given in 97 selected cases during the first week after the accident. Embolism was found in 10 cases, and pneumonia in 44 (27%). A myocardial infarction occurred during hospital treatment in 8 cases. Mortality was 9%, 7% in 150 operated cases, and 31% in the conservatively treated group. It varied in the different groups of additional diseases. Some new information for better medical therapy in cases of femoral neck fracture was obtained.

Fracture of the femoral neck is one of the commonest fractures in aged persons. Usually it appears at the age when many associated diseases are also present, and the death rate is therefore relatively high (1–4). The associated diseases may also hinder the proper rehabilitation of the patients, and so the treatment lasts many months.

Since there are very few systematic studies on diseases associated with femoral neck fracture and on its medical and anaesthesiological complications, the authors have tried to study a series of unselected cases.

MATERIAL AND METHODS

The material consists of 166 patients with fracture of the femoral neck or of the trochanter who were on treatment in 1966–68. The number of female patients was 135 and of males 31. The mean age was 71.9 (17–93) years. In 20 cases the accident had occurred in other hospitals or in nursing homes. The mean duration of hospital treatment was 32 days. The material was analysed with a computer (by Juha Kesonen, M. A., the Computer Centre of Helsinki University), and the statistical method used was Student's *t*-test.

Examination of the patients and preoperative therapy methods

In addition to the routine surgical examination, all the patients were studied personally by one of the authors. On the first day after admission the chest X-ray, ECG (12 leads), Hb, leucocytes, urine, serum potassium, creatinine, thrombotest and capillary blood gas analysis (Astrup) were studied in all cases. The respiratory function was studied with Wright's Peak Flow Meter. Its normal values were determined earlier in 33 normal persons, and the lowest value, at over 70 years of age, was 230 l/min. Breathing exercises were given by medical gymnasts to all whose peak flow value was under 200. To all patients aged over 60, and to patients with heart failure, digoxin was given perorally or intramuscularly. The medical condition of 150 patients was so good that the surgeons were allowed to operate upon them. All cases were monitored by cardioscope during anaesthesia, and pathological changes were registered by ECG.

From 7 to 10 days after operation or admission a new chest X-ray and ECG were taken. Twenty-one patients were excluded from the series due to quick transport to other hospitals or for lack of follow-up study. One of them died suddenly of pneumonia a few days after operation.

Sodium warfarin was given as anticoagulant to 97 patients.

RESULTS

Additional diseases were found in 145 of the 166 patients. The most important were heart failure in 72, myocardial infarction in the history of 14, and in the first ECG in 27 cases. Hypertension, with diastolic value over 100 mmHg, was found in 24, aortic stenosis in 21, other valvular

Table I. *Additional diseases in connection with fracture of the femoral neck*

	Other disease present	Without other disease
No. of cases	145	21
Duration of hospital treatment, days	44	71[a]
Mean peak flow value, l/min	194	337
Alkalosis, BE > 3.5, %	31	5[b]
Anticoagulant treatment, %	57	59
Complications during anaesthesia, %	60	33[b]
Pulmonary embolism during treatment, %	7	0
Pneumonia, %	28	19
Fresh heart infarction, %	6	0
Mobilization at hospital, %	69	86
Death rate, %	10	0

[a] $p < 0.01$.
[b] $p < 0.05$

disease in 6, rheumatoid arthritis (clinical finding) in 13 and diabetes mellitus in 16. Pulmonary emphysema as a clinical or roentgenological finding was present in 16 and changes due to other pulmonary or pleural diseases were seen in 43. Elevated serum creatinine (over 1.6 mg/100 ml) was found in 4 and lowered serum potassium (under 3.6 mEq/l) in 4. The normal base excess value in the Astrup analysis was ±2.5. Alkalosis

Table II. *Comparison of patients with and without heart failure*

	With heart failure	Without heart failure
No. of cases	72	94
Aortic stenosis, %	21	6[a]
Rheumatoid arthritis, %	4	11
Pulmonary emphysema, %	4	14
Other pulmonary diseases, %	17	33
Peak flow l/min, mean	182	236[a]
Alkalosis, %	28	28
Myocardial infarction changes in ECG, %	22	12
Atrial fibrillation, %	15	5
Digitalized patients, %	100	73
Anticoagulant treatment, %	72	48
Operated patients, %	90	90
Pulmonary embolism, %	7	5
Pneumonia, %	29	24
Fresh myocardial infarction, %	6	4
Mobilization at hospital, %	61	79[a]
Death rate, %	11	7

[a] $p < 0.05$

Table III. *Patients with and without previous myocardial infarction*

	With myocardial infarction	Without known infarction
No. of cases	27	139
Heart failure, %	59	40[a]
Diabetes mellitus, %	19	8[a]
Peak flow l/min, mean	153	224
Digitalized cases, %	96	83
Operated cases, %	81	92
Fresh myocardial infarction, %	7	4
Death rate, %	15	8

[a] $p < 0.05$.

(BE over 3.5) was found in 46, and acidosis (BE under −3.5) in 3. The changes in the first ECG were infarction in 27, extrasystoles in 16, and other changes in 62. The peak flow value as a mean of all cases was 212 l/min.

Method of operation and anaesthesia. Of the 166 patients 150 were operated on, using the Austin Moore prosthesis in 37 cases, and nailing in the others. The findings during anaesthesia are published in Honkonen et al. (6).

Findings after operation or during conservative treatment. During hospital treatment 118 patients were mobilized. The mean time after the accident was 20.4 days. Pulmonary embolism was diagnosed in 10 cases and pneumonia in 44 (27%). A fresh myocardial infarction was found in 8, right ventricular strain in ECG in 5 and rhythm disturbances in 11. Fifteen patients died during hospital treatment (9%), 38 were transferred to other hospitals, and 113 were discharged home.

Additional diseases were found in 145 cases; none was found in 21 cases. In addition to the usual medical diseases reported here, psychiatric and malignant illnesses were also present. Table I shows the most important comparative findings in these patients. The operation, the anaesthesiological methods and the duration of the operation were in comparable proportions in both groups.

Cardiac insufficiency was found in 72 cases, and in 94 it was not found. A comparison of these cases is presented in Table II. There were no significant differences in the duration or methods of anaesthesia and operation.

Previous myocardial infarction had occurred in 27 cases, and in 139 it was not found in the ECG. Table III shows the comparison between these patients. There was no difference in the percentage of anticoagulant therapy but the duration of anaesthesia and operation were significantly shorter in the myocardial infarction group. No differences were seen in the frequency of embolism and pneumonia.

Changes due to pulmonary disease except emphysema, were seen in 43 cases, and were not found in 123. The mean breathing function value was significantly lower (146 l/min) than in the comparison group (235 l/min). ECG changes (excluding infarction and rhythm disturbances) were found significantly more often (51 %) than in cases without pulmonary changes (33 %). There were no differences in the method of treatment or in the number of complications, but new pneumonia was found significantly more often (42 %) than in the comparison group (21 %). The mortality rate was the same in both groups (9 %).

Pulmonary emphysema was found in 16 patients. Mortality in these cases was 6 %.

Hypertension was found in 24 cases, non-hypertensive patients totalled 142. The serum potassium was lowered in 8 % of the hypertensives and in 1 % of the other patients. The difference is significant. Alkalosis was found in 42 and 25 % respectively. There also were more ECG changes in hypertensives, and anticoagulants were given to 63 % of them. Eighty-eight % of the hypertensive patients were operated on, using general anaesthesia in 63 % and spinal anaesthesia in 25 %. There was no difference in other complications, and no new myocardial infarctions were found among hypertensives. Mortality rates were 8 % and 9 % respectively.

Aortic stenosis was found in 21 cases. They were treated by the same methods as the comparative group and showed an equal number of complications. The mortality was higher (19 %) than among the other patients (8 %).

Rheumatoid arthritis was clinically verified in 13 cases. Two patients were treated with corticosteroids. No difference in complications and in the moment of mobilization were found. No deaths occurred in this group.

The significance of differences in the laboratory test values was studied only for the alkalosis and breathing function tests.

Table IV *Significance of the peak flow values in 154 cases*

	Peak flow l/min			
	1–99	100–199	200–299	over 300
No. of cases	14	47	59	34
Operated cases, %	93	96	90	91
General anaesthesia, %	7	26	75	79
Spinal anaesthesia,	71	53	10	1
Complications during anaesthesia, %	79	74	47	47
Pulmonary embolism,	0	4	5	6
Pneumonia, %	29	30	22	24
Fresh myocardial infarction, %	7	2	5	6
Death rate, %	0	11	5	9

Alkalosis, in most cases metabolic, was found in 46, and in the remaining 120 cases the Astrup test was normal or acidotic. An additional disease was found in 98 % of the alkalotic patients and in 83 % of the others, which is a significant difference. The mean breathing value was 187 among the alkalotics and 222 in the comparative group. The death rate was 9 %.

Respiratory function was measured in all cases. In 12 cases the result was 0, and so 154 cases gave confident values. The function was lower in women, in the aged patients, in cardiac insufficiency and in pulmonary diseases. Table IV shows the most important data.

Operated patients, numbered 150, and 16 patients were treated conservatively. There was no difference between these groups in the number of additional diseases. The duration of hospital treatment was 34 and 78 days, respectively, this being a significant difference. Embolism was found in 5 % of the operated and in 19 % of the non-operated patients ($p = < 0.05$). Anticoagulants were given to 50 % of the conservatively treated patients. New myocardial infarction was noted in 4 % and 13 % respectively ($p = < 0.05$). Right ventricular strain occurred in 4 % of the operated and 13 % of the non-operated patients ($p = < 0.05$). There was no difference in the moment of mobilization. The death rates were 7 and 31 % ($p = < 0.01$).

The Austin-Moore endoprosthesis was placed at operation in 37 and a nail in 113 patients. The moment of mobilization was the 19th day

in the former and the 21st day in the latter group. The death rate was equal.

Anticoagulants were given to 97 patients, and 69 were treated without them. Heart failure was found significantly more frequently in 54% in the former than in the latter group, 29% ($p = < 0.05$). There was no difference in the number of blood bottles transfused at operation or in the frequency of myocardial infarctions.

Pneumonia, seen in chest X-rays taken 7–10 days after operation or admission, was present in 44 cases (27%) and was not found in 122 cases. The mean age of the pneumonia patients was higher ($p = < 0.01$) There was no difference in the additional diseases. Breathing function was at the beginning of the hospital treatment significantly lower 183 l/min, in the patients with pneumonia than in the other cases, 223 l/min. Anticoagulants were given to 75% of the former and to 52% of the latter group ($p = < 0.01$). The operation was done under general anaesthesia in 45% before the onset of pneumonia, and in 54% of the other cases. Twenty per cent of the pneumonia patients, and 5% of the other patients died in the hospital ($p = < 0.01$).

Deaths Fifteen patients, aged 47–93 years, died in the hospital. Autopsy was performed on 11 of them. The cause of death of 4 non-autopsied patients was pneumonia in two cases, and myocardial infarction with insufficiency in the other two cases. Five pulmonary embolism cases were found among the 11 autopsied patients. Two of them were on anticoagulants, and the thrombotest level was under 25% during treatment. Three patients died of pneumonia and cardiac insufficiency one of fat embolism, one of myocardial infarction, and in one case heart muscle degeneration and pulmonary oedema were found.

DISCUSSION

Fracture of the femoral neck is an important consequence of the accidents of older age, and the prognosis is poor in many cases. The mortality has been earlier reported to be *c.* 8% (4) and 23% (9). The factors affecting the mortality are an associated disease, the long time of inactivity with increased risk of thromboembolism and pneumonia (8, 10), the anaesthesia and operation, and the complications of surgical and medical therapy It seems that, in order to shorten the hospital stay and to attain mobilization as early as possible, operative treatment is to be recommended. There is no reason to operate in an emergency because the prognosis is not better in such cases (7 8). The elective surgery allows the internist more time to check and treat the additional diseases, and the prognosis is thus better (7). The present study presents one possible system for examination and treatment of patients with fracture. There is reason to point out that, e.g., it was not possible to give anticoagulant therapy to all patients owing to lack of laboratory facilities. Another fact shown by this study is the high frequency of pneumonia during hospital treatment, revealed by systematic chest X-ray studies. The effort should be made to conduct breathing exercises also after operation in spite of a shortage of medical gymnasts.

The mortality in surgery of the aged is high (1 2), and often myocardial infarction is found (3 5). This study revealed several diagnostic groups in which the complication and the mortality varied very much. The total mortality of this group was 9% which is clearly lower than in another series (9) during a similar one-month duration of treatment. The present series was younger in age, however The mortality in operated cases was significantly lower (7%) than in conservatively treated cases (31%). The reasons for treating patients conservatively were a poor general condition, fresh heart infarction, cardiac aneurysm, prolonged pneumonia, psychiatric reasons, or refusal of operation by the patient or relatives. In the present series the operation was as far as possible selective, and the preoperative treatment and methods of anaesthesia were also selective. However it is possible to see the cases with the poorer prognosis in this series, namely those with an old myocardial infarction and with aortic stenosis, but the prognosis was only slightly poorer for the patients with heart insufficiency when using the treatment methods described. The prognosis seemed to be similar in cases with pulmonary changes or emphysema, hypertension or alkalosis. No patients with rheumatoid arthritis died. Measurement of the breathing function with Wright's Peak Flow Meter is a practical method for the study of bedridden patients. The peak flow value had less prognostic value in the present series, in which the anaesthesiological method

was chosen accordingly However it can help to avoid the use of general anaesthesia in cases with a poor respiratory function.

In the treatment of fracture of the femoral neck in a surgical hospital, the internist and the anaesthesiologist may help greatly in the diagnosis and treatment of complicated cases. The cases with a poor prognosis can also be found more easily Digitalization, early systematic use of anticoagulants, and wider use of breathing exercises may to some extent improve the usually poor prognosis.

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Table I. *The incidence of additional diseases in the various anaesthesia groups, as percentages of the number of cases in the respective group*

Additional disease	General (86 cases)	Spinal (50 cases)	Combined spinal + general (14 cases)
	Type of anaesthesia		
Heart insufficiency	41	50	36
Myocardial infarction verified in ECG	9[a]	20[a]	29[a]
Atrial fibrillation	*11*	*10*	*14*
Aortic stenosis	14	10	14
Other valvular disease	2	6	7
Hypertension (diastolic pressure over 100 mmHg)	17	12	0
Pulmonary emphysema	2[a]	14	36[a]
Other pulmonary disease (TB, pneumonia, pleuritis, bronchiectasia)	16[b]	40[a]	57[a]
Diabetes	5[a]	20[a]	7

[a] $p<0.05$
[b] $p<0.01$

Moderate hypotension, fall of 30 to 50% of the rest value.

Severe hypotension, the blood pressure fall was over 50% of the rest value.

In the comparison of complications during and after surgery special attention was paid to the patient's age and additional preoperative diseases.

RESULTS

Concurrent diseases

Preoperatively 21 patients were healthy the rest had one or more additional diseases. In the general anaesthesia group 83% of the patients had some additional disease, in the spinal anaesthesia group 94% and in the combined group 100% were sick. The difference between the general anaesthesia and the two other groups is statistically highly significant.

The condition of the patients in the different anaesthesia groups is shown in Table I.

It appears that the patients in the general anaesthesia group had earlier myocardial infarcts, emphysema and diabetes less than the two spinal groups, and that the difference is significant.

The general anaesthesia group also had markedly less other pulmonary diseases than the other groups, and this difference is highly significant.

As an explanation it must be remarked that the type of anaesthesia was determined individually from case to case. Spinal anaesthesia was the most common choice if the patient's condition was very poor especially if the respiratory function was deemed inadequate. For instance, the mean respiratory peak flow was 271 l/min in the general anaesthesia group, 142 l/min in the spinal group, and 144 l/min in the combined anaesthesia group.

Duration of anaesthesia and operation

The mean duration of the anaesthesia was 146 min and the mean duration of the operation 99 min. Table II shows the durations in the different anaesthesia groups. The durations were shortest in the spinal group and longest in the combined group these differences are higly significant. Spinal anaesthesia was never chosen in the first place if the operation was estimated to last over two hours.

In the combined group the operation turned out to be more difficult than expected, which explains the long mean duration of anaesthesia and surgery in this group When the material was split into groups according to the duration of the anaesthesia (less than 120 min, 57 cases 120–180 min, 64 cases and more than 180 min, 29 cases), it was found that there was no significant difference in relation to the additional preoperative diseases in the groups.

Complications during operation

The complications consisted of hypotension and different kinds of disturbances in the heart rhythm.

Fifty-six patients, mean age 69 years, were without complications during surgery and 80% of them had some concurrent disease besides the

Table II. *The mean duration of anaesthesia and operation in minutes in the various anaesthesia groups*

	General (86 cases)	Spinal (50 cases)	Combined spinal + general (14 cases)
Duration of anaesthesia	144	126[a]	183[a]
Duration of surgery	101	80[a]	142[a]

[a] $p<0.01$

femoral neck fracture. Complications were re corded in 94 patients, mean age 74 years 93% of them had some additional disease. The dif ferences are significant.

In the group without complications 34% had cardiac insufficiency and 5% aortic stenosis, and the mean respiratory peak flow was 256 l/min. In the group with complications these percentages were 49% and 17% respectively and the mean respiratory peak flow was 193 l/min. The dif ferences are significant.

Patients with low respiratory peak flow values were especially prone to complications. When the series was split into groups according to the peak flow values (Table III), a clear correlation between low values and complications during anaesthesia was seen. The difference between the low value groups and the higher value groups is significant.

In Table IV are seen the complications in the different anaesthesia groups. In the general anaesthesia group 51% of the patients had complications, in the spinal group 78% and in the combined group 79% the differences are statistically significant.

The incidence of moderate hypotension was significantly higher in the spinal group This was probably to be expected, since spinal anaesthesia per se tends to lower the blood pressure and since the spinals were done on patients whose general condition was very poor In the combined group, moreover the procedure was drawn out, as dif ficulties occurred in the surgical work.

Severe hypotension occurred in 13% of the patients with earlier hypertension, in contrast to an incidence of 1% in the patients who were normotensive before surgery The difference is significant.

Cardiac insufficiency patients had significantly

Table III. *The incidence of complications during anaesthesia in the groups with different respiratory peak flow values, as percentages of the respective group*

	Respiratory peak flow l/min			
	<100 (14 cases)	100–199 (47 cases)	200–299 (59 cases)	>300 (34 cases)
Complications during anaesthesia	79	74	47	47

Table IV *The incidence of complications during anaesthesia in the different anaesthesia groups, as percentages of the number of cases in the respective group*

	Type of anaesthesia		
Type of complication	General	Spinal	Combined spinal + general
Severe hypotension (4 cases)	1	6	0
Moderate hypotension (26 cases)	13	28*	14
Slight hypotension (57 cases)	31	44	57
Disturbances of heart rhythm (18 cases)	12	12	14

* $p<0.05$.

more slighter hypotension (53% versus 38%) than patients with adequate heart function. Even valvular disease and earlier attacks of extrasystoles predisposed to slight hypotension (9% ver sus 1% and 18% versus 6% respectively these differences are highly significant).

In the ECG tracings there appeared traces of earlier infarctions, conduction block of various degrees, extra systoles and atrial flimmer Disturbances of the heart rhythm were recorded in 18 patients, 16 of whom had had the same type of disturbance before the anaesthesia. The two new cases were an 82 year-old woman with chronic rheumatoid arthritis, and a woman aged 80 with valvular disease and cardiac insufficiency

The complications during anaesthesia were non fatal and in no case alarming, and were controlled by ordinary therapeutic measures, i.e. vasopressors, blood tranfusions, and in some cases hydrocortisone.

Complications during recovery

I the period of recovery from anaesthesia 6% of the general anaesthesia group, 14% of the spinal group and 14% of the combined anaesthesia group had complications. The complications consisted of heart rhythm disturbances (12 cases), depressed ST segment in the ECG tracing (14 cases), and persisting hypotension that had to be treated (4 cases). As could be expected, more heart rhythm disturbances were recorded in pa tients who had had earlier heart

lies.

Table V *The incidence of postoperative complications in the various anaesthesia groups as percentages of the number of cases in the respective group*

Postoperative complications	Type of anaesthesia General	Spinal	Combined spinal + general
Pneumonia	23	28	50*
Pulmonary artery embolism	5	4	7
New heart infarction	3	6	0
New right ventricular strain in the ECG	2	2	0
New rhythm disturbance	9	6	0
Death	3	10	14

* $p<0.05$

There was no significant correlation between the length of anaesthesia and the complications in the recovery period.

Postoperative complications

The cases with complications in the postoperative period, the nature of the complications and their distribution among the different anaesthesia groups are recorded in Table V

Postoperatively 41 patients had pneumonia. The percentage in the combined anaesthesia group is especially high, 50% and the difference from the other groups is significant.

There is no correlation between postoperative pneumonia and preoperative low respiratory peak flow values or emphysema. Pulmonary artery embolism was more common *in the patients who* had had moderate hypotension during surgery: 12% of these, but only 3% of the other patients had postoperative pulmonary artery embolism the difference is significant.

The overall mortality was 9% in the whole series (operated + conservatively treated cases), and the distribution among the different anaesthesia groups was 3%–10%–14% The low mortality rate in the general anaesthesia group differs significantly from the rates in the other groups. Of the 15 cases of death 5 were non-operated and 10 operated.

The causes of death were: pulmonary artery embolism (5 cases), pneumonia and cardiac insufficiency (5 cases), myocardial infarction and cardiac insufficiency (3 cases), cardiac insufficiency and pulmonary oedema (1 case), and fat embolism (1 case)

There was a correlation between concurrent diseases and mortality 100% of the patients who died had had some additional disease, i.e. there

ciency and pulmonary oedema (1 case), and fat embolism (1 case)

DISCUSSION

It is a well known fact that the surgical risk increases with age (3 4, 8 11). Age in itself is not the deciding factor but the patients concurrent diseases, which become more *common* in the elderly influence the outcome (3 11). In the present series as well the additional diseases were numerous, 87% of the patients having one or more concurrent diseases. Some authors are of the opinion that there is a clear correlation between the length of operation and the post *operative complications and mortality (2, 8, 11).* Topkins and Artusio (10) for instance, express as their opinion that no operation in the age group over 65 years should last more than three hours. In spite of the best anaesthetic management, these patients deteriorate with time.

Other investigators, on the other hand, could *not find that a long operation time increased the* rate of postoperative complications or of mortality (3 10).

The present study seems to corroborate this view There was no significant correlation between a long operation time and complications during and after surgery The 50% frequency of pneumonia *during the postoperative period in* the combined anaesthesia groups is an exception. This group contained patients who were in a poor condition preoperatively difficulties were encountered in the surgical management of the patients, and the operation time was highly significantly longer than in the other groups. It may possibly be argued *that, since the mean anaesthesia* time even in this group was only 183 min, our operation times were not of such a length that these correlations with intra- and postoperative complications would have become apparent.

The importance of a careful preoperative examination and treatment of additional diseases is repeatedly stressed in *the literature (3 6, 7 8).* The elderly patient should never be operated on

as a dejour case, for this leads to very poor results (6, 8).

According to many authors the type of anaesthesia does not seem to influence the results (1 2, 3 10). Spinal analgesia was earlier recommended, especially for patients with pulmonary insufficiency because it interferes less with the patient's own breathing. In a semi-high spinal anaesthesia the oxygen tension of the blood does not fall, instead, there is a fall in the p CO_2 both during and after the operation (6).

When comparing the different anaesthesia groups in this series, one should constantly keep in mind that the type of anaesthesia was chosen from case to case.

Following the reasoning above, spinal anaesthesia was chosen especially for patients who were in a poor general condition, especially if the pulmonary status was bad.

Earlier myocardial infarction frequently predisposed us to use spinal analgesia too. It is thus not surprising that there are more complications, both during and after surgery in the two spinal groups than in the general anaesthesia group

During anaesthesia the older patients had more complications than the younger ones (mean age in the complications group was 74 years, in the non-complications group 69 years), especially if they had a concurrent disease. Cardiac insufficiency aortic stenosis, hypertension and pulmonary disease predisposed the patient to complications during surgery The spinal groups had more complications than the general anaesthesia group, and the most common complication was hypotension. This is natural, since spinal anaesthesia leads to sympathetic denervation and thus to a lowering of the blood pressure.

Even in the recovery period the spinal anaesthesia groups had a higher incidence of complications, especially heart rhythm disturbances and depressed St segment in the ECG than the general anaesthesia group. This probably reflects the low oxygen tension that is common in aged patients after surgery (9), even when oxygen is administered.

As this also mirrors the fact that the spinal groups contained the patients who were in the poorest condition preoperatively we have to second the view that the type of anaesthesia is not in itself a decisive factor as regards the outcome of the operation. The higher mortality in the spinal groups, in our opinion, was due to the same cause.

The age of the patients and their concurrent diseases were more important.

Tentatively we offer the opinion that the respiratory peak flow value, which most closely followed the incidence of complications, could perhaps be used as a means to predict the latter

Even when no value or a poor value was obtained, owing to lack of co-operation on the part of the patient, this clearly mirrored the patient's feebleness and debility thus predicting the overall result.

Important factors leading to complications in surgical patients are preoperative cardiac insufficiency aortic stenosis, hypotension and pulmonary insufficiency All our patients withstood surgery well, with the exception of the four with grave hypotension, and we feel that this is due to the careful preoperative examination and the promptly started treatment of concurrent disease. On the other hand, we had a very high rate of postperative pneumonia (23%–28%–50% in the different anaesthesia groups) these were in part clinically silent" and were only detected by systematic postoperative chest X-ray examination.

Many authors have stressed diminished ventilation as the most important cause of postoperative complications in the aged (3 6). Renck (9) recently reported a very low oxygen tension in these patients postoperatively This has also been regarded as the principal cause of postoperative ECG disturbances (11).

It was surprising that we could find no correlation between postoperative pneumonia and preoperative emphysema and low respiratory peak flow values. This is in discordance with other studies (3 11).

In any case this series clearly is an indication for more active postoperative care. The best policy would perhaps be to concentrate these patients to a separate ward, where the patients could be put through a uniform breathing exercise programme. In this way in our opinion, many of the postoperative pneumonias would be voided.

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CARDIOVASCULAR EFFECTS OF GLUCAGON

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Abstract. Glucagon, 50 μg/kg, was given as bolus injection into the pulmonary artery to ten patients with severe heart disease. In all patients an increase in cardiac output was found, those with highest initial output increasing most. Oxygen consumption was unaltered, systemic vascular resistance decreased, potassium levels fell, serum glucose and insulin levels rose. No effect was observed on heart rate. In four other patients the same registrations were made after infusion of 90 ml 30% glucose with 8 IU insulin. No effect on cardiac output was observed, although glucose levels increased and potassium levels fell to the same extent as after glucagon injection. In the same patients the cardiac output increased after glucagon injection. The investigation supports previous reports that glucagon might be a useful therapeutic agent in low output failure.

Glucagon has for a long period been known for its ability to increase blood sugar and has an established rôle in the treatment of hypoglycemia. During the last years several workers (5, 6, 9) have reported that glucagon also has positive inotropic and chronotropic effects on the heart. How this effect is mediated is still unknown. Activation of the adenylcyclase system with increased formation of 3',5' cyclic adenosinemonophosphate (cyclic AMP) probably plays an important role. As it is commonly believed that stimulation of adrenergic beta-receptors is mediated through an increase in cyclic AMP, it has been suggested that glucagon acts through the beta-receptors (12). Blocking of the beta-receptors, however, does not abolish the inotropic effect of glucagon (4). The effect also seems to be independent of whether digitalis glycosides have been used or not.

Several studies of the actions of glucagon on the human circulation have been reported (1, 5, 6, 9). The present work was carried out in order to study the qualitative and quantitative aspects of the action of glucagon in a group of patients with severely impaired cardiac reserves.

MATERIAL AND METHODS

Ten patients, five women and five men, were studied in connection with diagnostic right heart catheterization (Table I). Six patients had myocardial disease (group I), five primary myocardial disease, and one coronary heart disease. Four patients had different kinds of valvular disease (group II). The age varied from 39 to 71 years, average 59 years. All patients were on digitalis glycosides, seven had atrial fibrillation, and all belonged to functional class III or IV of the New York Heart Association classification.

All subjects were investigated in the fasting state. An ordinary right heart catheterization was done. Thereupon glucagon (Glucagon NOVO-freeze dried glucagon hydrochloride in vial + solvent), 50 μg/kg, was given as a bolus injection into the pulmonary artery. Different parameters were registered before and at intervals after glucagon injection. Intravascular pressures were measured in the femoral artery, right atrium, pulmonary artery and in pulmonary artery wedge position. Heart rate and ECG were recorded. Blood samples were drawn from the pulmonary artery and femoral artery for determination of insulin (8), growth hormone (14), free fatty acids, glucose, potassium and oxygen saturation. Pressure recordings were made with Elema Schönander transducer EMT 35 no. 2439 through Cournand catheters nos. 6 or 7 in the right heart, and Stille teflon catheter no. 30-8103-14 in the arteries. The pressures were recorded with an Elema Schönander Mingograf no. 81. Cardiac output was determined by indicator technique using indocyanine green and Beckman Cardio Densitometer. Automatic integration of the curve was performed by the instrument. The linearity of the downward slope of the curve was tested on logarithmic paper when doubt concerning the applicability of the Stuart Hamilton formula arose. In six patients direct measurement of the oxygen consumption was performed.

In further four patients (Table I, group III), two with primary myocardial disease, and two with coronary heart disease, the effect of an isolated rise of blood sugar and insulin on cardiac output was studied during similar catheterization. During a period of 15 min 90 ml 30% glucose and 8 IU soluble insulin were infused intravenously. Forty-five minutes after the glucose-insulin infusion was started, glucagon was given. The same registrations were made as in groups I and II.

Table I. *Clinical features of patients studied*

PMD = primary myocardial disease. CHD = coronary heart disease. MS = mitral stenosis. MI = mitral insufficiency. AI = aortic insufficiency. TI = tricuspidal insufficiency. DAP = persistent ductus arteriosus. op. = operated. F br = atrial fibrillation. Flu. = atrial flutter

Group	Pat. no.	Sex	Age	Diagnosis	Functional class (NYHA)	Heart rhythm	X-ray heart volume (ml/m²)
I	1	♂	58	PMD	III	Sinus	590
	2	♂	39	CHD	III	Sinus	730
	3	♂	64	PMD	III	Fibr	1 040
	4	♀	56	PMD	III	Fibr	840
	5	♂	69	PMD	IV	Sinus	720
	6	♀	53	PMD	III	Fibr	760
II	7	♀	71	MS, MI, AI, TI	IV	Fibr	1 580
	8	♀	60	MS op., MI TI	IV	Fibr	—
	9	♀	63	AI op., MI op.	III	Fibr	900
	10	♂	55	DAP AI	III	Fibr	640
III	11	♂	50	MI op., CHD	IV	Sinus	645
	12	♂	48	PMD	II	Sinus	570
	13	♂	59	PMD	III	Flu.	800
	14	♂	53	CHD	II	Sinus	630

RESULTS

Fig. 1 shows the effect of glucagon on cardiac output. In all patients an increase was observed, the average maximal increase being 1.28 l/min or 36% of the pre-glucagon value. The rise is statistically significant ($p < 0.01$). The increase varied considerably from 0.3 to 3.0 l/min. In group I the cardiac output increased on an average 41% while the average in group II was 25% (Table II). Comparison of the patients with cardiac output lower than 3.5 l/min with those who had a higher initial cardiac output showed that the latter increased their cardiac output on an average 47% against 27% for the former group. This difference is even greater when the absolute increase is compared. The patients with the best

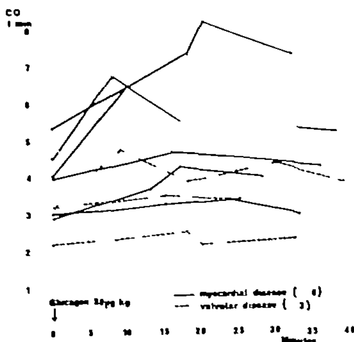


Fig. 1. Effect of single injection of glucagon on cardiac output in nine patients.

Table II. *Cardiac output before and maximal increase after injection of glucagon*

Group	Pat. no.	Cardiac output before (l/min)	Max. increase (l/min)	Max. increase (%)
I	1	4.5	[illegible]	51
		3.0	0.4	13
	3	4.0	0.7	18
	4	5.3	3.0	57
	5	2.9	1.4	48
	6	4.8	[illegible]	63
II	7	3.2	1.6	50
	8	[illegible]	0.3	14
	9	3.[illegible]	0.3	9
Mean			1.21	36

myocardial performance, therefore, showed better ability to respond to glucagon.

The maximal increase in cardiac output occurred between 8 and [illegible]4 min (mean 15 min) after the injection of glucagon, and the effect lasted more than 25 min in seven cases. Cardiac index increased from an average of 1.56 before glucagon injection to 2.6[illegible] after the injection.

Systemic vascular resistance (Fig. [illegible]) fell in all patients except one ($p < 0.01$). The heart rate did not increase significantly; there was no change in systemic blood pressure, pulmonary artery pressure or pulmonary artery wedge pressure. The oxygen consumption was also unaltered, and there was no change in the pulmonary arteriolar resistance (Table III).

Right ventricular stroke work was found to increase significantly except in two patients with valvular heart disease (Fig. 3). We were not able to plot these data into ventricular function diagrams, because pulmonary artery wedge pressures were not representative of left ventricular diastolic pressure in the cases with valvular disease, and because right ventricular pressures were not measured at the time of maximal effect. Right atrial

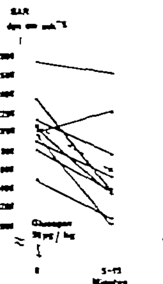


Fig. [illegible]. Systemic vascular resistance (SAR) after glucagon injection.

pressure recorded during withdrawal of the catheter was therefore correlated to right ventricular stroke work (Fig. 4). This is possibly not representative of the changes during maximal drug effect, although it indicates a positive inotropic even at this time.

Serum potassium fell from an average value of 3.94 mEq to 3.20 mEq after 20 to 30 min. Blood sugar increased on average 56%, and insulin concentration increased 150% (Fig. 5). Information concerning free fatty acids, growth hormone and insulin are listed in Table IV.

After glucose-insulin infusion (group III) the blood sugar increased by 116% and the serum potassium fell by 17% (mean values). The cardiac output did not change significantly (Table V), but increased on an average 27% after glucagon in the same series.

No serious complications were observed after the injection of glucagon. Four patients had nausea and vomiting 1 to 5 min after injection; this

Table III. *Unchanged hemodynamic parameters* ($p > 0.05$)

	Heart rate	Oxygen cons. (ml/min)	Fem. art. (mmHg)	Pulm. art. (mmHg)	Pulm. art. wedge (mmHg)	Pulm. art. res. (dyn cm sec^{-5})
No. of patients ...	10	8	10	10	7	6
Before glucagon (mean)	68	234	135/73–100	26	18	237
After glucagon (mean)	7[illegible]	225	139/73–98	25	20	[illegible]

Table I. *Clinical features of patients studied*

PMD = primary myocardial disease. CHD = coronary heart disease. MS = mitral stenosis. MI = mitral insufficiency
AI = aortic insufficiency TI = tricuspidal insufficiency DAP = persistent ductus arteriosus. op. = operated.
Fibr = atrial fibrillation. Flu. = atrial flutter.

Group	Pat. no.	Sex	Age	Diagnosis	Functional class (NYHA)	Heart rhythm	X-ray heart volume (ml/m²)
I	1	♂	58	PMD	III	Sinus	590
	2	♂	39	CHD	III	Sinus	730
	3	♂	64	PMD	III	Fibr	1 040
	4	♀	56	PMD	III	Fibr	840
	5	♂	69	PMD	IV	Sinus	720
	6	♀	53	PMD	III	Fibr	760
II	7	♀	71	MS, MI, AI, TI	IV	Fibr	1 580
	8	♀	60	MS op., MI, TI	IV	Fibr	—
	9	♀	63	AI op., MI op.	III	Fibr	900
	10	♂	55	DAP AI	III	Fibr	640
III	11	♂	50	MI op., CHD	IV	Sinus	645
	12	♂	48	PMD	II	Sinus	570
	13	♂	59	PMD	III	Flu.	800
	14	♂	53	CHD	II	Sinus	630

RESULTS

Fig. 1 shows the effect of glucagon on cardiac output. In all patients an increase was observed, the average maximal increase being 1.28 l/min or 36% of the pre-glucagon value. The rise is statistically significant ($p < 0.01$). The increase varied considerably from 0.3 to 3.0 l/min. In group I the cardiac output increased on an average 41% while the average in group II was 25% (Table II). Comparison of the patients with cardiac output lower than 3.5 l/min with those who had a higher initial cardiac output showed that the latter increased their cardiac output on an average 47% against 27% for the former group. This difference is even greater when the absolute increase is compared. The patients with the best

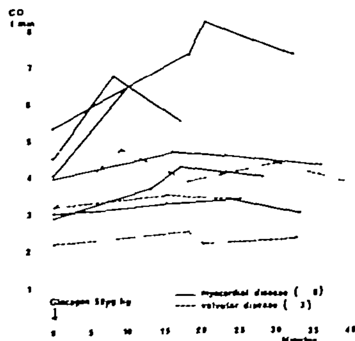


Fig. 1. Effect of single injection of glucagon on cardiac output in nine patients.

Table IV *Effect of glucagon on free fatty acids, growth hormone and insulin*

	Before glucagon			5–15 min			20–30 min		
Pat. no.	FFA	GH	Insulin	FFA	GH	Insulin	FFA	GH	Insulin
2		6.2	7.5		5.0	>98.0		8.2	50.0
3		4.0	38.5		8.4	>91.0		2.2	71.5
4	990.0	3.2	8.8	1 340.0	7.7	21.6	970.0	7.0	32.8
5	680.0	14.2	78.2	640.0	6.7	84.5	480.0	7.2	45.6
6	870.0	16.4	<4.9	1 030.0	4.8	77.4	960.0	7.0	32.8
7		6.6	<4.9		>20.0	< 4.9		>20.0	19.2
8	1 380.0	9.2	<4.9	1 470.0	10.7	26.0	1 030.0	20.0	6.9
9	1 100.0	5.6	<4.9	890.0	14.9	11.3	800.0	21.0	11.5
10	630.0	14.0	47.5	520.0	8.6	75.5	380.0	10.6	73.0
Mean	936.0	8.8	22.2	981.0	9.6	55.2	770.0	11.4	35.0

Free fatty acids, µmol/L.
Growth hormone, ng/ml.
Insulin, µU/ml.

that these reserves are smaller the more severe the myocardial impairment is (11).

In contrast to other workers (3 5 6 9 13) we have not found any positive chronotropic action of glucagon. This may partially be explained by the fact that seven of the patients investigated had atrial fibrillation. However no definite difference between patients in atrial fibrillation or sinus rhythm was found.

A significant decrease in systemic vascular resistance was found. This is probably due at least partially to a selective dilatating effect of glucagon on the mesenteric arteries (10).

The maximal rise in blood sugar occurs later than the maximal inotropic effect, and it is therefore unlikely that the rise in blood sugar is the main cause of the effect of glucagon on cardiac output. This is confirmed by the finding that infusion of glucose-insulin had no effect on cardiac output, in spite of an increase in blood sugar

Table V *Effect of glucose-insulin (g–i) and glucagon on cardiac output in four patients (group III)*

Pat. no	Initial value (l/min)	Max. increase during g–i infusion (l/min)	Max. increase after glucagon (l/min)
11	3.8	−0.3	1.2
12	4.7	0.8	1.4
13	2.8	0.8	0.2
14	3.5	0.6	—

of 116% whereas the subsequent glucagon injection caused a definite increase of the cardiac output in the same patients.

The glucagon injection caused marked and statistically significant changes in blood glucose, insulin and potassium levels, whereas the changes in other electrolytes, free fatty acids and growth hormone were variable, inconstant and statistically insignificant. The change in blood glucose occurred relatively late and is readily explained by the well known effect of glucagon on liver glycogenolysis. The plasma insulin levels reached maximum and started to decline before the peak glucose concentration was reached. The increase is probably due to a combination of the direct effect on insulin release and an indirect effect due to the rise of blood glucose. The fall in serum potassium was marked, and in some cases levels below the lower normal limits were reached. The fall may be explained by the increase serum insulin combined with hyperglycemia, and is reproduced by glucose-insulin infusion. Low serum potassium disposes to cardiac arrhythmias, especially in digitalized patients. In no case, however were adverse changes in cardiac rhythm observed in connection with the injections.

Although all patients in this study were fully digitalized, the increment of cardiac output after glucagon injection is of the same magnitude as in undigitalized animals or humans (2, 7). This supports the concept that the inotropic action of digitalis and glucagon is mediated by independent mechanisms.

The present study confirms that the failing human heart may benefit from intravenous glucagon. It indicates that glucagon might be a useful therapeutic agent in acute depression of heart function, for instance cardiogenic shock in the course of myocardial infarction, or heart failure following cardiopulmonary bypass.

Evaluation of its therapeutic value in these conditions is going on in several centers, and the reports so far are promising.

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UROKINASE INHIBITORS IN SERUM IN A CLINICAL SERIES

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Abstract. In order to find out whether the content of inhibitors of the urokinase activation of plasminogen in serum varies to such an extent as to be of any significance in the treatment of thrombosis with urokinase, these inhibitors have been assayed in 1 008 patients with different types of liver diseases, renal diseases, thrombosis, leukaemia, cancer, myelomatosis, pregnant women, and women on low dose gestagen therapy. The level of urokinase inhibitors varied widely. Very high levels were, above all, found among the patients with renal diseases, especially acute uraemia, after kidney transplantation and in malignant diseases. Thus it is obvious that patients who might require fibrinolytic treatment differ widely from one another regarding inhibition not only of the effect of streptokinase but also that of urokinase.

According to recent literature (6, 16, 17) urokinase is preferable to streptokinase for inducing thrombolysis. This conclusion is based on the following grounds. Streptokinase is antigenic, and the blood contains streptokinase-neutralising antibodies in quantities varying widely from one individual to another. This means that the amount of streptokinase necessary for obtaining optimal fibrinolysis varies widely from one patient to another. Urokinase, on the other hand, does not stimulate antibody formation, and it has been proposed that it may be given in a standard dose to all patients (17).

In order to find out whether the content of urokinase inhibitors varies to such an extent as to be of any significance in the treatment of thrombosis with urokinase, it was decided to study the records of patients referred to our laboratory and in whom urokinase inhibitors had been assayed.

In several of the patients we determined not only the urokinase inhibitors, but also the two types of antiplasmin, the immediate reacting antiplasmin (α_2-macroglobulin) and the slow-reacting antiplasmin. The investigation was therefore extended to include a comparison of these three inhibitors for any relative variation with type of disease.

MATERIAL AND METHODS

The material consisted of sera from 1 008 patients with the diagnoses given in Table I.

Determination of inhibitors of urokinase activation of plasminogen in serum (urokinase inhibitors). A clot method previously described by Paraskevas et al. (15) was used. To 0.5 ml serum (diluted 1:4, 1:5, 1:10, 1:20) the following substances were added in the order given: 0.1 ml urokinase (Leo Pharmaceutical Products, Ballerup, Denmark, 5 500 Ploug U/mg, in dilution 200 Ploug U/ml), 0.1 ml plasminogen (Grade A, freeze dried, 15 Sgouris CU/mg protein, AB Kabi, Stockholm, in dilution 0.01 mg/ml), 0.2 ml bovine fibrinogen (AB Kabi, Stockholm, dilution 0.5%) and 0.1 ml thrombin (Topostasin®, Roche, Basle, Switzerland, dilution 20 N.I.H. U/ml). The dilutions were made with 0.9% NaCl. The lysis times were measured and the inhibitory effect of the samples was expressed in per cent of a standard consisting of pooled serum from 25 healthy persons. Two blanks were used in which 0.5 ml of 0.9% NaCl and 0.5 ml of 0.1% ε-aminocaproic acid solution (EACA), respectively, were added. EACA was used to check the results of tests performed on different occasions. Normal range 60–140%.

α_2-macroglobulin (α_2M) ("the immediate antiplasmin") was determined according to an esterolytic method by Ganrot (3). Normal range is 80–120%.

Antiplasmin ("the slow reacting antiplasmin") was determined according to a fibrin plate method previously described by Ekelund et al. (2). Normal range is 60–140%.

RESULTS

Liver diseases

A. *Urokinase inhibitors* were determined in 304 patients with different types of liver disease. The material is distributed according to diagnoses in Table I. The miscellaneous group (35 patients) in-

Table I. *Diagnoses distribution and number of cases with low (<60%), normal (60–140%) and high (>140%) values of the inhibitors of plasminogen activation*

Diagnoses	Inhibitors of plasminogen activation (no. of cases) Low <60%	Normal 60–140%	High >140%	Total no. of cases
Liver diseases	5	206	93	304
Cirrhosis	5	131	37	174
Hepatitis	0	15	7	22
Primary cancer	0	12	12	24
Secondary cancer	0	31	19	50
Miscellaneous	0	17	18	35
Renal diseases	0	16	93	109
Chronic uraemia	0	11	59	70
Acute uraemia	0	0	14	14
Renal and urinary tract diseases without uraemia	0	5	5	10
After renal transplantation	0	0	15	15
Thrombosis	2	118	28	148
Leukaemia	7	83	54	144
Chronic	0	45	28	72
Acute	7	38	26	72
Other malignant diseases	1	40	35	76
Myelomatosis	15	113	30	158
Pregnancy	13	27	0	40
Contraceptive agents (low dose gestagen)	1	21	7	29

cluded unclassified jaundice, hepatomegaly of obscure origin, biliary diseases, pancreatitis (one case), acute yellow liver atrophy (one case), carbon monoxide poisoning with liver injury (one case) and hepatic coma (one case)

The content of urokinase inhibitors was normal in 206 of the 304 patients, high in 93 (>140%) and low in 5 (<60%) 75.3% of the patients with liver cirrhosis had normal values of urokinase inhibitors and 21.4% values >140%. Among the patients with primary cancer of the liver 50% had high values and 50% normal while only 38% of the patients with secondary cancer of the liver had high values. The value was normal in the patient with pancreatitis and in the one with yellow liver atrophy but increased in the patient with carbon monoxide poisoning and in the one with hepatic coma.

B. *α_2-macroglobulin* was determined in ten patients with liver disease (eight with liver cirrhosis, two with secondary cancer) The level was normal in six, including five with normal and one with increased urokinase inhibitors (403%). In the remaining four all of whom had a normal content of urokinase inhibitors, the α_2M was increased (mean 172%) All these four patients had liver cirrhosis.

C. *Antiplasmin* was determined in 25 of the patients. It was normal in 23 and somewhat increased (162% and 146%) in the remaining two. These two had liver cirrhosis and a normal content of urokinase inhibitors.

Renal diseases

A. *Inhibitors of urokinase* were determined in 70 patients with *chronic uraemia* of varying origin. The activity proved normal in 11 and increased in the remaining 59 No difference in this respect was found between those who had been treated conservatively and those treated with dialysis (mean values 208% and 229%). Fourteen patients with *acute uraemia* had markedly increased values (mean 316%). Of ten patients with *renal or urinary tract diseases without uraemia* the inhibitor activity was increased (mean 197%) in five and normal (mean 122%) in the remaining five.

In 15 patients who had undergone *renal transplantation* the inhibitor level, which was followed for up to two years after the operation, was in-

variably raised (mean 456%) throughout the observation period.

B α_2-*macroglobulin* was determined in 35 patients with *chronic uraemia*. In most (19) of them it was normal (Table II). The level was low in nine and elevated in seven. In the patients with the low level the serum creatinine was markedly increased. Of ten patients with *acute uraemia* five had normal values, four low (< 80%) and one a high value (13-year-old girl). In the patients who had undergone *renal transplantation* the α M was low namely 44–92% (mean 77%).

C. *Antiplasmin* was determined in ten patients with *chronic uraemia*. They all had normal values (mean 102%). Seven patients with *acute uraemia*, in whom antiplasmin was assayed, likewise had normal values (mean 95%). Antiplasmin was also determined in all the patients who had undergone *renal transplantation* and was found to be normal (mean 103%).

Thrombosis

A. *Inhibitors of urokinase* were determined in 148 patients with phlebographically verified thrombosis. The examination was carried out at least three months after the acute phase of the disease. The content of urokinase inhibitors proved to be normal in 118, increased in 28 (> 140%) and decreased (< 60%) in two

B $_2$-*macroglobulin* was determined in 108 of the 148 patients with thrombosis. It was normal in 85 low in 20 and slightly elevated in only three (132% 136% and 176% respectively). In none of the patients with increased α_2M were the inhibitors of urokinase increased.

C. *Antiplasmin* was determined in 93 of the 148 patients with thrombosis. It was normal in 87 and increased in four (> 140%). In none of the 94 patients examined were the inhibitors of urokinase increased. In two the antiplasmin was low (< 60%). In both of these patients the inhibitors of urokinase were normal, while in one also the α_2M-value was low (53%).

Leukaemia

Inhibitors of urokinase were determined in 144 patients with various sorts of leukaemia (72 chronic and 72 acute). All 144 patients had received some form of treatment. The level was normal in 83 raised in 54 and low in 7 The distribution of lymphatic and granulocytic forms of leukaemia was the same among the patients with increased content of inhibitors of urokinase. In all the patients in whom the urokinase inhibitor level was low the white blood cell count was normal or low

Table II. *Mean values of inhibitors of plasminogen activation, α_2M and antiplasmin in renal diseases, thrombosis and pregnant women*

Diagnoses	Inhibitors of plasminogen activation Mean ( )	α_2M Mean (%)	Anti-plasmin Mean ( )
Renal diseases			
Chronic uraemia	203	100	102
Acute uraemia	316	93	95
After renal transplantation	456	77	103
Thrombosis	117	99	99
Pregnant women	79	121	157

Other malignant diseases

Inhibitors of urokinase were determined in 76 patients with malignant tumours. In 35 of them the inhibitors were increased and in 40 they were normal. Strikingly high values up to > 500% were found in some patients with widespread metastases in the skeleton and other tissues.

Myeloma

Urokinase inhibitors were determined in 158 patients with myeloma. The values were found to be low in 15 normal in 113 and increased in the remaining 30.

Pregnant women

Inhibitors of fibrinolysis were determined in 40 women in the third trimester of pregnancy

A. *Inhibitors of urokinase* The level was low (< 60%) in 13 women and normal (mean 79%) in the remaining 27

B. α_2-*macroglobulin*, on the other hand, proved to be somewhat increased with a mean of 1 1%. In 18 the value was > 120% and in 21 it was normal. In one woman it was low (74%).

C. *Antiplasmin*. Also the antiplasmin was increased during the third trimester (mean 157%). In 30 it was > 140% and in the remaining 10 normal.

Women using oral contraceptives

Urokinase inhibitors were determined in 29 women using oral contraceptives (low dose gestagen) The value was normal in 21 and moderately raised in 7 in only one of them was it more than 200 % In the remaining women the value was low (25 %).

DISCUSSION

In the present clinical material the level of in hibitors of the urokinase activation of plasminogen was found to vary widely

In most (131 of 174 cases) of the patients with liver cirrhosis the level of the urokinase inhibitors was normal. In the 24 patients with primary liver cancer the level was normal in only half in the other half it was raised. Broadly speaking, liver injury not due to cancer does not appear to af fect the level of the urokinase inhibitors appreci ably

The possibility of an increased amount of urokinase inhibitors playing a role in the causation of thrombosis has been discussed (11 14). In this connection it might be pointed out that only in 28 of our 148 patients w th thrombosis was the inhibitor content increased. In order to avoid recording reactive increases of the urokinase in hibitors, the patients were not examined until at least three months after the acute attack. We therefore do not feel that an increased content of these inhibitors in the blood is a common cause of thrombosis. Also other inhibitors of fibrinolysis were largely normal.

In 59 of the 70 patients with uraemia, due to chronic renal disease, urokinase inhibitor content was increased. As known (1 7) in patients with chronic uraemia the fibrinolytic activity in the cir culating blood is decreased. The role played by the kidneys in this respect has been discussed by various authors (8 18) Investigations by us (5 10) however suggest that the kidney does not play a specific role, but rather that the low fi brinolytic activity is due to the markedly increased content of inhibitors of fibrinolysis. In the treatment of patients with renal insufficiency by dialysis, thrombi often occur in the shunts. Such treatment, therefore, includes the use of fibrinolytics, e.g. streptokinase or urokinase. The usually high content of urokinase inhibitors in these patients should thus be borne in mind when deciding the dose of these preparations. This also applies to patients who have undergone renal transplantation, in whom the increase of inhibitors is marked, as it was in all 15 in the present material. It is remarkable that the α_2M tended to be low in patients with uraemia, and especially in those who had undergone renal transplantation (mean value 77 %). In our opinion this argues clearly in favour of the two inhibitors being of a different nature and showing different reaction patterns.

In most cases with leukaemia the content of inhibitors was not influenced by the fundamental disease (it was normal in 83 and increased in 54) No difference was found between the granulocytic and lymphatic forms of leukaemia. No over representation of acute leukaemia was found in the group with an increased content of inhibitors. The finding of low inhibitor values among patients with leukaemia and a low or relatively low number of white blood cells in the circulating blood was noteworthy Practically all of the pa tients had received some sort of treatment As the material was collected during a relatively long period (from 1962 to 1969) the method of treat ment was not uniform.

It is known that urokinase inhibitor content increases in the presence of reactive processes (4), such as different types of malignant diseases. We found an increase of inhibitors in 35 of 76 patients with malignant disease, while the value was normal in 40 patients. In most cases the increase was moderate, about 200 % which is that most commonly seen in reactive processes. Some of our patients showed a special coagulation pattern with, inter alia, a markedly increased amount of urokinase inhibitors (> 500 %), a very high content of fibrinolytic split products in serum and no fibrinolysis in the blood.

Compared with the other groups of diseases, myeloma was common among the patients with a low urokinase inhibitor content. In 30 patients the content was increased and in the remaining 113 normal. Myeloma is also often associated with fibrinolytic activity in the blood (9) which may be at least partly explained by the low content of in hibitors of plasminogen activation.

Among 40 pregnant women the urokinase inhibitor content was rather low (mean value 79 %). During the latter part of pregnancy the fibrinolytic activity decreases while plasminogen, factor VIII, prothrombin, fibrinogen increase (12) The de-

creased content of inhibitors of plasminogen activation may constitute a mechanism for compensating these changes. The other inhibitors of fibrinolysis, α M and antiplasmin, on the other hand, were increased during the latter part of pregnancy

We found it interesting to note that the inhibitor content differed so little from normal in women using oral contraceptives (low dosage gestagen). Nilsson and Kullander (13) showed that the urokinase inhibitors decreased 20–30% during combined oestrogen-gestagen therapy

As mentioned above, our findings suggest that the various inhibitors of fibrinolysis, α_2M, antiplasmin and plasminogen activation vary independently of another in various diseases.

Thus it is obvious that patients who might require fibrinolytic treatment differ widely from one another regarding inhibition not only of the effect of streptokinase but also that of urokinase This applies in particular in renal insufficiency after renal transplantation and in malignant diseases. In these cases one can never be sure whether a standard dose of the fibrinolytic substance (urokinase) is unnecessarily large, optimal or too small. From our results it is obvious that in these cases there is great risk of the inhibitor content being increased, tendency which, in our opinion, should be borne in mind before treatment is started.

ACKNOWLEDGEMENT

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THE DIAGNOSTIC VALUE OF SERUM LDH ISOENZYMES AND HEAT STABLE AND UREA-STABLE LDH MEASUREMENTS

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Abstract. Serum heat-stable and urea-stable LDH measurements have been found to detect acute myocardial infarction more sensitively than the separation of LDH_1 or LDH_2 *isoenzymes with agar gel electrophoresis. On the* other hand, serum heat-stable and urea-stable LDH tests give better information of myocardial damage if the serum LDH pattern was confused by simultaneous augmentations of liver-originated LDH isoenzymes. The simple heat-stable and urea-stable LDH measurements thus seem to give more accurate confirmation of myocardial damage than the more complicated electrophoretic separation of LDH isoenzymes.

The confirmation of organ-specific diagnosis has been improved considerably by the electrophoretic separation of serum LDH isoenzymes. In particular the diagnosis of myocardial infarction has gained in certainty (5–28, 31–53). This is due to the preponderance *of the fast moving LDH isoenzymes* (LDH_1 and LDH_2) in the myocardium, quite unlike other tissues except kidney and red blood cells (45–52). The measurement of serum LDH *isoenzymes allows confirmation or exclusion of* liver or skeletal muscle damage if suspected simultaneously with myocardial infarction, since LDH_5 isoenzyme characterizes these tissues (50–52). In order to escape the tedious electrophoresis and the staining of the bands, several simplified methods for the determination of LDH isoenzymes have been introduced (7). The chief clinical use has been for the determination of serum heat-stable (4, 9, 18, 34, 42, 43) and urea-stable (12, 25, 29, 39) LDH activities. These two methods have been adjusted to measure the activity of fast moving LDH isoenzymes (13, 24, 26, 35, 47, 49–51) and might theoretically be expected to give similar information of myocardial damage to that obtained from the electrophoretic separation of LDH isoenzymes. Since these simple tests seem promising in the detection of myocardial damage, the evaluation of their clinical usefulness, compared with electrophoretically separated LDH isoenzymes, was considered to be worth studying.

MATERIAL AND METHODS

There were 30 patients with acute myocardial infarction, 21 males and 9 females. The mean age was 59.0 years. The onset of the infarction was estimated to have occurred with the onset of severe substernal pain. The diagnosis was based on the clinical picture, leukocyte count, elevated body temperature, increased sedimentation rate and serial 12-lead electrocardiograms. Furthermore, routine analyses of serum aspartate aminotransferase (GOT) and α-hydroxybutyrate dehydrogenase (HBD, ref. *19) were made on the first three days after admission.* In all except two patients pathological *Q* waves with simultaneous ST segment changes were observable. In one of these two patients small *q* waves with negative symmetrical *T* waves and in another only negative symmetrical *T waves at the III and aVF leads developed in* serial electrocardiograms, indicating equivocal inferior cardiac infarction. In these patients neither the clinical picture nor lung scanning or chest X-ray indicated pulmonary embolism.

For the detection of congestive heart failure the patients were examined by us daily for rales in the lungs, by palpation of the liver, observations of edema in the ankles and back, and auscultation of heart sounds for mitral incompetence. The blood pressure and heart rate were determined. If pulmonary congestion was suspected, chest X-ray was taken. In order to obtain further support for liver damage, serum alanine aminotransferase (GPT) and ornithine carbamoyltransferase (OCT, ref. 20) measurements were made on the first four days in hospital.

In seven patients liver injury was encountered on the above mentioned grounds. The clinical evidence of con-

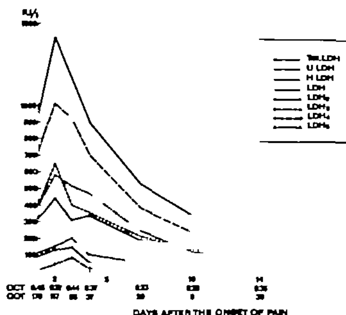


Fig. 4. Daily serum LDH parameters in a patient who, on admission, was defibrillated and resuscitated for ventricular fibrillation evoked by a large myocardial infarction. Serum GOT and OCT activities also shown.

enzymes were often elevated (Fig. 6). If myocardial infarction had been suspected in these patients, the heat stable and the urea-stable LDH tests would have been false-positive in five and six cases, respectively but the LDH_1 and LDH_2 isoenzymes in 12 and 11 cases, respectively. The heat-stable LDH activity was pathological in two patients with infectious hepatitis, in two patients with calculus in the bile duct, and in one patient with liver cirrhosis. The urea-stable LDH activity was pathological in a further patient with infectious hepatitis.

In 11 patients with signs suggestive of acute pulmonary embolism the serum total LDH activity was abnormal in only *four* cases. LDH_1 and LDH_2 were increased in three patients, and LDH_3 in four but LDH_3 isoenzyme, the most characteristic isoenzyme of the pulmonary tissue (52), in one patient only (Table I).

The depression of total LDH activity by heat and urea as compared with LDH isoenzyme bands is shown in three representative patients in Fig. 7. Heat depresses the total LDH activity considerably more than urea in the methods used. The

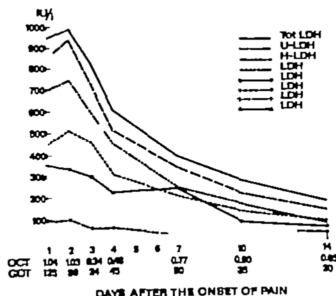


Fig. 5. Daily serum LDH parameters in patient with severe congestive heart failure. The liver congestion was observable already on admission.

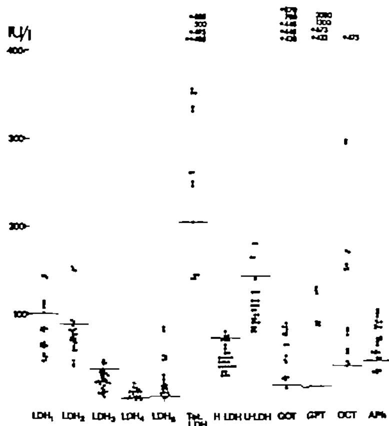


Fig. 6. Serum enzyme activities in 32 patients with liver disease. Activities expressed in IU/l serum, except OCT for which 100 times greater values are used to render them comparable with the other activities. Horizontal lines indicate the upper normal limits.

total activities differ from each other in these two methods, due mostly to the different temperatures and pyruvate concentrations used in the methods.

DISCUSSION

The sensitivity and the prolonged elevation of the activity in the post-infarction phase make the determination of serum LDH isoenzymes superior to other enzyme tests in the detection of myocardial infarction (5 ...8, 31 45 53). It was confirmed in the present study that the simple heat stable and urea-stable LDH measurements were in this respect as good as, or even better than, the more complicated electrophoretic separation of LDH isoenzymes. This was evident whether

Table I. *Serum enzyme activities (IU/l) in 11 patients with acute pulmonary embolism. Pathological activities in italics*

Pat. no.	Total LDH	LDH$_1$	LDH$_2$	LDH$_3$	LDH$_4$	LDH$_5$	H-LDH	U-LDH	HBD	GOT	GPT	OCT
1	180	74	83	16	2	5	55	115	120	*19*	14	0.40
2	145	88	48	7	0	2	60	85	85	10	10	0.28
3	*295*	*165*	*103*	18	0	*9*	*160*	*215*	*205*	*23*	*33*	0.09
4	175	75	81	15	2	2	55	120	110	17	11	0.22
5	*215*	74	84	*41*	*6*	*10*	55	125	—	16	*32*	0.28
6	175	75	56	26	5	*13*	30	95	110	13	12	*0.45*
7	185	88	78	15	0	4	*75*	*155*	130	*30*	*28*	0.53
8	175	77	61	33	2	2	70	*145*	*150*	12	7	0
9	*200*	*106*	*99*	34	5	*16*	*95*	*175*	*180*	*24*	*20*	*0.58*
10	*270*	*143*	*111*	16	0	0	*90*	*195*	*240*	*38*	*18*	*0.43*
11	145	81	48	16	0	0	50	100	90	9	13	0.35

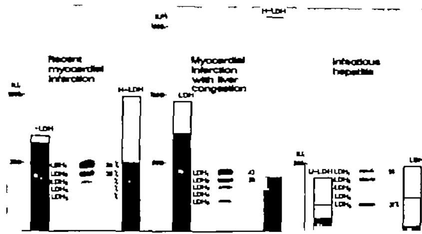


Fig. 7 Serum urea-stable and heat-stable LDH activities compared with LDH electrophoretic runs in three representative patients. The height of the columns shows the total activity by the two methods. The black part of the columns shows the U-LDH or H-LDH activity The numbers within the black part indicate the percentile share of U-LDH and H-LDH of the corresponding total activity and the horizontal lines the upper normal limits.

LDH_1 LDH_2 or $LDH_1 + LDH_2$ isoenzymes were used as indicators of myocardial damage.

Any method with apparent sensitivity to detect myocardial damage must be evaluated also in respect of its specificity In clinical practice liver injury in the form of liver congestion, pulmonary infarction and skeletal muscle damage are the most important diseased conditions to be taken into consideration in such a test. Due to the dissimilar organ distribution of LDH isoenzymes their separation can be expected to help the differentiation of myocardial infarction from these conditions (45 52) In clinical work, however the differentiation of pulmonary embolism from myocardial infarction by means of LDH isoenzymes seems to be of questionable value (3 5 14 30). This was observed also in the present few cases of pulmonary embolism with all the LDH tests studied.

When congestive heart failure occurs in the acute post-infarction period, the confirmation of myocardial damage by means of serum enzyme tests may be complex or confusing. Liver injury seems to be common after myocardial infarction, as verified by the augmentation of serum OCT (7) this enzyme test being considered specific for liver damage (37 38). In this period heart-originated and liver-originated serum LDH isoenzymes may be abnormalized simultaneously (1). This was the situation in the seven patients in whom congestive heart failure appeared in the acute post-infarction phase. The myocardial damage could be confirmed in these patients despite the superimposed LDH_5 increase, and the heat-stable and urea-stable LDH tests gave clearer information of the infarction than the LDH_1 or LDH_2 isoenzyme determinations.

If myocardial infarction had been under consideration in the 32 patients with liver disease, false-positive results would have been obtained more rarely with the serum heat-stable or urea-stable LDH tests than with the LDH_1 or LDH_2 isoenzyme determinations. This is because, simultaneously with LDH_5 isoenzyme, such amounts of LDH_1 and LDH_2 isoenzymes are released into the blood that it is impossible to rule out the possibility of myocardial damage. Serum heat-stable and urea-stable LDH measurements allow the exclusion of this possibility considerably better

When compared with other tests the serum LDH isoenzyme measurements and, as supported by the present study heat-stable and urea-stable LDH tests seem so far to be the most specific tests for the detection of myocardial infarction. These diagnostic tools seem to be of greater accuracy than the determinations of serum HBD (11 21 40) and creatine kinase. The latter is sensitively abnormalized in skeletal muscle disease (8, 16) and myocardial damage (6 22 41 47), although not in liver disease (32) or in the shock (27). Recently serum creatine kinase has been reported to rise also in some cases of pulmonary embolism (33). In the postoperative phase serum creatine kinase may be released from skeletal muscle into the blood (36), and then the superimposed rise caused by myocardial damage is usually impossible. The determination of serum LDH isoenzymes may then be of distinctive diagnostic value in revealing myocardial infarction (17 18). Similarly the determination of serum LDH

isoenzymes may allow one to exclude myocardial damage if it is suspected on the basis of serum creatine kinase, total LDH or GOT elevations after electroconversion (23). Further information on organ specificity by means of creatine kinase isoenzymes seems so far to be of limited clinical value (10, 44). It must be emphasized, however that in complicated clinical conditions the electrophoretic separation of LDH isoenzymes may give more information than the heat-stable or urea-stable LDH measurements, at least when it is combined with simultaneous serum OCT and creatine kinase measurements. And it must be remembered that all the studied LDH tests show increased serum activity in megaloblastic anemia, hemolytic states and renal cortical damage.

The heat-stable LDH test seems to be more sensitive than the urea-stable LDH test in the detection of myocardial damage, although the actual activities are greater in urea-stable measurements. A disadvantage of heat-stable LDH measurements is that calculation of the values close to normal limits may be sometimes uncertain. This is due to the denaturation of serum proteins, which makes the serum turbid and the progress of the reaction uneven. This is not seen in the urea-stable LDH measurements, which in addition are slightly more simple to carry out.

ACKNOWLEDGEMENT

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POST TRANSPLANT HYPERCALCEMIA DUE TO MOBILIZATION OF METASTATIC CALCIFICATIONS

An Alternative to Tertiary Hyperparathyroidism

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Abstract. A case of transient hypercalcemia in 29-year old female, who received renal transplant from her homozygotic twin-sister has been examined by 47 calcium tracer study and traditional calcium and phosphorus balance techniques. Parathyroid activity was evaluated by measuring the tubular reabsorption of calcium and the tubular maximum capacity of glucose. The study revealed pronounced negative balance due to pronounced hypercalcuria and low intestinal absorption of calcium in functionally hypoparathyroid patient. It is concluded that the hypercalcemia was caused by mobilization of metastatic calcifications deposited in the terminal phase of uremia and during hemodialyses. It is suggested that other calcium metabolic disturbances may have similar pathophysiological mechanism, including some cases of post-transplant hypercalcemia, which have hitherto invariably been considered to be due to so-called tertiary hyperparathyroidism.

Hypercalcemia is a well-known phenomenon following successful renal transplantation (13, 14). Although the hypercalcemia has been shown to be of a transient nature in the majority of cases, it seems to be generally accepted that the hyper calcemia in all patients is due to hyperfunctioning of the parathyroid glands, or so-called tertiary hyperparathyroidism (1 4). That alternative explanations for a transient post-transplant hyper calcemia should be taken into consideration is illustrated by the present study of a young woman who appeared to be functionally hypoparathyroid during a post-transplant hypercalcemia, and in whom the transient rise of serum calcium was in all probability due to a mobilization and gradual elimination of metastatic calcifications deposited during the terminal phase of uremia.

CASE REPORT

The patient was 29-year-old married female with an 11-year history of chronic glomerulonephritis, verified histologically by biopsy in 1957 and by bilateral nephrectomy in 1967. In 1962, 1963 and 1965 she had three normal pregnancies and deliveries. Through 1965–66 she developed deterioration of renal function with an accompanying severe arterial hypertension. After initial conservative therapy peritoneal dialysis was started in February 1967 and in May of the same year she was transferred to regular biweekly hemodialysis program using Scribner shunt and 2-layer Kill-dialyzer. During this phase she developed severe uremic polyneuropathy. Due to the severe renal hypertension, nephrectomy on the left and right side was undertaken in May and October 1967 respectively. The surgery was complicated by infections, and not until late 1967 was she considered apt for transplantation. On January 30, 1968, renal transplantation was carried out from homozygotic twin-sister. The postoperative course, illustrated in Fig. 1 was complicated by leakage and later by relative stenosis of the ureteral anastomosis, which required reoperations on February 6 and 15 (in Fig. 1 indicated by I and II). The renal function, as measured by the endogenous clearance of creatinine, showed fluctuations concurrently with the surgical complications, thereafter gradual improvement; by the end of April 1968 the glomerular filtration rate (GFR) was almost normal (50 ml/min), and in July 1969 1 / years after transplantation, she was normotensive, had normal renal function (clearance of creatinine 68 ml/min), and the neuropathy had regressed. The fall of GFR after the first reoperation was initially considered possible immunological rejection, for which reason treatment with prednisone was initiated (25 mg 4 daily), until after two days repeated tissue-typing and extensive examination of red cell, leucocyte and thrombocyte groups confirmed the identity of the twin-sisters.

Serum calcium and serum phosphorus levels

During the terminal phase of conservative treatment the levels of serum calcium and phosphorus in our patient

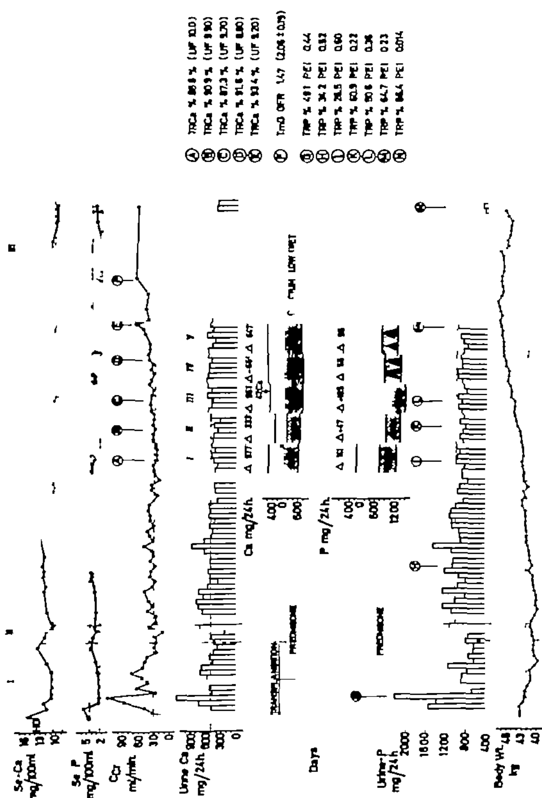


Fig. 1 Course of events after transplantation, indicating the serum levels, urinary excretions, and balances of calcium and phosphorus. The fecal (hatched) and urinary (blank) excretion per day of the tw charted according to Raffenstein et al.

were characterized by low normocalcemia and hyperphosphatemia (average 8.9 and 10.0 mg/100 ml, respectively), the pattern usually found in patients with terminal uremia. Through the three months of peritoneal dialysis slight normalization of these disturbances took place (average serum calcium and phosphorus 9.6 and 6.9 mg/100 ml, respectively). With regard to the levels seen during the phase of hemodialysis it should be mentioned that until May 1968 the dialysis base fluid in our department had calcium content of approximately 10 mg/100 ml. Accordingly patients undergoing dialysis would develop hypercalcemia during treatment having on average 10.8 before and 12.9 mg/100 ml after dialysis. This is well known as the hard water syndrome (7). The present patient had serum calcium levels considerably higher than average, 14 and 16 mg/100 ml before and after dialysis, respectively. Possibly these high values were due to an immobilization caused by the polyneuropathy.

The patient' serum phosphorus levels ere kept at the upper level of normal predialytically (5.9 mg/100 ml) and at the lower level of normal postdialytically (2.7 mg/100 ml) on peroral treatment with aluminium hydroxide suspension in the inter-dialysis period, treatment given throughout the phase of hemodialysis.

The level of alkaline phosphatase activity was within normal.

After transplantation she developed transient hypercalcemia of approximately three months duration, whereafter normalization took place. Control of the serum calcium levels in the following eighteen months consistently showed normocalcemia. Due to the longstanding hypercalcemia it was decided to undertake study of the calcium metabolism ith the aim of clarifying whether the hypercalcemia was of parathyroid or non-parathyroid origin. This study was undertaken one and half months after transplantation.

METHODS

The study was performed by means of 1) traditional chemical balance regime of calcium and phosphorus; 2) simultaneous tracer study with 47-calcium; and 3) indirect estimation of parathyroid activity by determinations of the renal tubular reabsorption of calcium (TRCa%) (22) and of the ratio between the maximal renal tubular reabsorptive capacity of glucose and the clearance of inulin (TmG/GFR) (9).

Balance regime

The patient was put on metabolic regime according to the principles advocated by Reifenstein et al. (20). After 20 days on diet well defined in regard to calcium, phosphorus, nitrogen and sodium, collections of feces, urine and parallelly prepared diet were started.

Feces were collected in periods of six days duration; marking of individual periods was carried out with carmine (1 g). Urine was collected in 24-hour portions in bottles containing 20 ml 10% hydrochloric acid to prevent precipitation of secondary calcium phosphates. The quantitative collection of urine was estimated by daily analysis of the 4-hour excretion of creatinine.

Diet was given in 3-meal system, and the composition of the fed diet was checked by analysis for calcium and phosphorus of the contents of plastic bucket fed parallelly with the patient. The dietary intake of calcium and phosphorus varied from 634 to 785 mg, and from 1 213 to 1 492 mg, respectively. The protein and sodium contents of the diet were kept constant, at level of 77 g and of 129 mEq daily respectively. During the investigation the patient was kept at an increasing degree of mobilization without exposure to direct sunlight.

Chemical methods

Analysis for calcium was carried out by complexometric titration with sodium ethylene diamine tetraacetate and using calcein as indicator (2). The fluorescence of the calcium-calcein complex was registered by EEL photocells and light spot galvanometer thus applying an objective estimation of the color shift.

Serum was titrated directly. The urine was acidified and titrated after addition of sodium citrate to the alkaline titration medium. Feces and diets were homogenized (Ultraturrax) after sufficient addition of hydrochloric acid (10%) to lower the viscosity of the samples. Aliquots of the homogenized portions of approximately 7 g were ashed at 600°C for 24 hours, and the ashings were extracted ith 10% hydrochloric acid up to total of 50 ml. This extract was then titrated in the same way as urine in aliquots of 50 and 100 μl under addition of sodium citrate to the titration medium.

The *phosphorus* in serum and urine was analysed according to the method of Goldenberg and Fernandez (8), and feces and dietary ash extract ere analysed by the same technique.

Creatinine in serum and urine was analysed according to the method of Hawk et al. (10).

Tracer technique

In the middle of the balance regime an intravenous dose of approximately 25 μCi 47-calcium chloride was given with specific activity of approximately 80 mCi g Ca (supplied by The Danish Atomic Energy Commission, Risø). In the following eight days samples of serum, urine and feces were counted to determine the decay curve for 47-calcium in serum and urine and loss of activity with urine and feces. Measurements of *serum 47-calcium* activity were performed on aliquots of 3 to ml serum taken several times during the first 4 hours and twice daily for the following seven days. *Urine* activity was measured in volumes of 5 ml after careful mixing of the total acidified diuresis. *Feces* activity was estimated by measuring the activity in weighed 5 ml aliquots of the total homogenized feces sample. The homogeneity of the total feces portion was checked by taking triple aliquots from different parts of the total portion. All aliquots were counted in well-type 3 3' NaI(Tl) crystal connected to an autogamma spectrometer (Packard) with lower level of 1 000 keV thus avoiding 47-scandium energy.

Treatment of isotopic data

The isotope data were calculated according tion of the method of Wendeberg (24).

Table I. *Balance data (mg/24 h)*

Balance period	Dietary calcium	Fecal calcium	Urinary calcium	Balance calcium	Dietary phosphorus	Fecal phosphorus	Urinary phosphorus	Balance phosphorus
1	609	746	440	−577	1 213	426	928	−141
2	664	571	426	−333	1 393	476	870	+ 47
3	747	767	530	−551	1 492	498	889	+105
4	73*	833	550	−651	1 410	542	923	− 55
5	648	765	530	−647	1 332	503	885	− 55

plies a model consisting of two compartments, rapidly (E_1) and a slowly (E_2) exchangeable pool of calcium. Our modification of the method refers to the time interval used for calculation of the accretion rate. Wendeberg considers day 5 (t_1) to day 10 (t_2) after the injection to be optimal to avoid influence of (*a*) delay of initial mixing of the isotope ver the two compartments and (*b*) possible return of tracer from calcified tissues with a rapid turnover rate to the exchangeable pool. Individual variation may be critical, however and we have therefore found it reasonable to individualize the choice of t and t_2. The decay curve of the specific activity in serum and urine has been computerized to yield the regression with the highest coefficient of correlation over at least a period of 72 hours between 24 hours and 168 hours after injection of the isotope. This approach has resulted in cur tailment of the experimental period as well as a reduction of the dose of radioactivity given. In the present patient t was chosen as t 42.25 hours, and t_2 as t 148 hours.

RESULTS

Serum calcium level

Immediately after the transplantation the serum calcium fell from the high (postdialytical) level to nearly normal values through the first three days (17 1 to 10.8 mg/100 ml) (Fig. 1). On the ninth day after the transplantation the serum calcium started to rise again to a maximum value of 13.1 mg/100 ml on the fourteenth day With the institution of steroid treatment it rapidly fell to almost normal levels, but rose abruptly on discontinuation of the drug to approximately 12.5 mg/100 ml, where it remained for a period of approximately one month. Following this a gradual decline to normal levels was observed in the course of one month. Control of the serum calcium levels in the following eighteen months has consistently shown normal values.

Balance study

Throughout the balance study the patient had an excessive excretion of calcium in the urine, aver aging approximately 500 mg/day Since the ex cretion of calcium with feces was also excessive the balance was markedly negative (Table I).

Like calcium, phosphorus was excreted in large quantities with the urine amounting to an average daily excretion in the balance period of approx imately 900 mg. Fecal excretion was, however not very high, and the resulting balance of phos-

Table II *The 47-calcium/balance data (period 4)*

	Our case (g)	Controls (g)	Reference
Rapidly exchangeable pool E_1	1.5456	2.20 ± 0.45 (1 S.D.)	Dymling, 1964 (6)
Slowly exchangeable pool E_2	1.5301	2.38 ± 0.79 (1 S.D.)	Dymling, 1964 (6)
Accretion rate (/24 h)	0.7841	0.449 ± 0.101	Dymling, 1964 (6)
		0.534 ± 0.270	Neer et al., 1967 (16)
Resorption rate (/24 h)	1.435	0.568 ± 0.253	Neer et al., 1967 (16)
Endogeneous fecal calcium excretion (/24 h)	0.159	0.167 ± 0.032	Neer et al., 1967 (16)
Intestinal absorption (/24 h)	0.058	0.360[a]	Nordin, 1968 (18)
Balance of calcium (/24 h)	−0.651	−0.012 +0.190	Nordin, 1960 (17)
Intestinal absorption % of dietary intake	8 1 %	20–60 %	Nordin, 1968 (18)

[a] 8 mg/kg bodyweight.

phorus was slightly negative or zero. The cumulative balances of calcium and phosphorus are illustrated in Fig. 2. It appears from the figures that a gain of bodyweight of 3.9 kg occurred during the balance period.

Tracer study

The results of the 47-calcium study are given in Table II together with the calculated value of "true" absorption, balance value, and the calculated value of resorption. It appears that the accretion rate was higher than in the control groups reported (6, 16, 17, 18), while endogenous fecal calcium and the rapidly and slowly exchangeable pools were normal. Intestinal absorption was found to be very low, being only 8% of dietary intake during the investigation. The resorption rate was high, the balance being markedly negative.

Measurements of parathyroid activity

Tubular reabsorption of calcium (TRCa%), which at the beginning of the balance regime was 86.6% 90.9 and 87.3%, rose to 91.6 and 93.4% at the end (*A* to *E*, Fig. 1). These values are distinctly below those found in hyperparathyroid patients (Fig. 3).

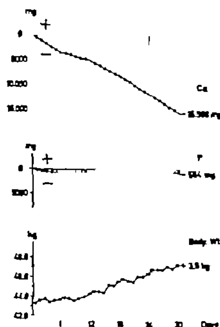


Fig. 2. Cumulative balance of calcium and phosphorus, and bodyweight during balance study.

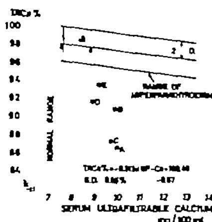


Fig. 3. Tubular reabsorption of calcium related to serum ultrafiltrable calcium. *A, B, C, D, E* refer to symbols used in Fig. 1. ● 18 cases of parathyroid hyperfunction; ○ our case.

The tubular maximal resorptive capacity of glucose per ml clearance of inulin (the TmG/GFR) was found to be 1.47, a value which is distinctly lower than the value of 2.06 ± 0.19 found in normal females (*F*, Fig. 1) (9).

The tubular reabsorption of phosphorus (the TRP%) showed a gradual, through somewhat fluctuating increase from low to finally normal values (*G* to *N*, Fig. 1) (19). The phosphate excretion index (the PEI) was abnormal with the exception of the last value.

DISCUSSION

In hypercalcemic disorders it is essential to settle whether the hypercalcemia is due to high secretion of parathyroid hormone or whether this secretion is low due to the negative feedback reaction to a raised serum calcium level. Hereafter further diagnostic procedures can be employed to reach the final correct diagnosis of the basal disturbance.

The optimal approach would be a direct determination of parathyroid hormone secretion rate. In the absence of such a method indirect measures for parathyroid hormone activity must be used.

In the present paper the main emphasis has been on the determination of the TRCa% and the TmG/GFR ratio, since previous studies have demonstrated that these measures are of value in

the differentiation of parathyroid and non-parathyroid hypercalcemias (9 22). In the present patient the TRCa% was found to be low as compared to the level of the TRCa% in hyperparathyroid hypercalcemia with an identical elevation of the ultrafiltrable calcium in the serum, indicating a hypercalcemia of non-parathyroid origin. The impression of the hypercalcemia as non-parathyroid is supported by the finding of a low TmG/GFR value. The significance of this has been published by Halver (9). A high ratio is found in hyperparathyroid hypercalcemia, a low in hypercalcemia due to non-parathyroid causes. The renal handling of phosphorus (PEI and the TRP%) was identical to that seen in hyperparathyroidism. Reduction of renal function, also when the reduction is only minimal, does, however invalidate the value of this measure in distinguishing between parathyroid and non-parathyroid hypercalcemias. The immediate suppressive effect of prednisone on the serum calcium levels also indicates that the hypercalcemia was of a non-parathyroid nature (5). Finally the finding of a *low* intestinal absorption of calcium in the hypercalcemic phase during the balance study may be taken as supportive evidence against a hyperparathyroid hypercalcemia, as many patients with hyperparathyroidism have a high intestinal absorption of calcium (15).

The most conspicuous features of the study were hypercalcemia, a pronounced hypercalcuria and a heavy negative balance of calcium. This loss of calcium must originate from sources within the body Potential sources are the bones and extraosseous calcifications. In non-parathyroid disorders a high resorption of calcium from the bones may be due to bone metastases or to acute immobilization. Our patient had no malignant disease, and she was in a state of increasing mobilization and anabolism with increase of bodyweight. It is therefore reasonable to assume that the calcium excreted from the body a total of 15 g during the balance regime, originated from extraosseous, so-called metastatic calcifications.

Metastatic calcifications are a well-known phenomenon in several pathological conditions. Some of these extraosseous calcifications are readily recognizable as part of a disturbed metabolism of calcium and/or phosphorus, some are not. The former include hypercalcemia of varying etiologies and conditions with hyperphosphatemia. That uremic patients are carriers of such calcifications is well known. The cause of their formation is thought to be the retention of phosphorus in late renal failure, with resulting precipitation of calcium phosphates in the soft tissues. Our patient did have such calcifications, as demonstrated before transplantation in the cornea and the conjunctival mucous membrane and during surgery as massive calcifications in the vessels.

After renal transplantation phosphorus will be readily excretable by the kidney and metastatic calcifications are gradually resorbed, resulting in a transient hypercalcemia and hypercalcuria. Such a hypothesis is in agreement with the findings in our patient, who presented evidence of hypercalcemia of non-parathyroid origin. The apparent discrepancy between the cumulative negative balance of calcium and the cumulative zero balance of phosphorus is readily explained by the gain of bodyweight with anabolic retention of phosphorus occurring in this period.

That resorption of metastatic calcification may give rise to transient hypercalcemia, and hypercalcuria has recently been documented (3) and has on earlier occasions been indicated by other groups to occur in sarcoidosis and in vitamin D intoxication (11 12). That they should be pathophysiologically active in various disturbances of calcium metabolism in patients with renal disease, a condition in which metastatic calcifications are a frequent finding, is not surprising. Rather it is surprising that their possible primary participation in several calcium metabolic disturbances has not been considered earlier One such disturbance might be hypercalcemia arising in the recovery phase *from acute renal failure* (21 23). *In this* condition direct estimation of parathyroid hormone activity in serum evidenced a hypoparathyroid state compatible with a hypercalcemia due to mobilization of metastatic calcifications following renal elimination of phosphorus retained in the oliguric phase.

The frequent occurrence of hypercalcemia and hypercalcuria following renal transplantation has hitherto invariably been considered as indicative of tertiary hyperparathyroidism despite the fact that in most cases the metabolic disturbance resolves spontaneously It seems probable that several of the transient post-transplant hypercalcemias may be of a similar nature to that in the patient described in the present study

It is therefore recommended that careful examinations be carried out to save the patients from unnecessary and potentially damaging surgical interference.

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BLOOD INORGANIC PHOSPHATE, PYRUVATE AND LACTATE DURING AN INTRAVENOUS GLUCOSE TOLERANCE TEST IN ISCHEMIC CARDIOVASCULAR DISEASE

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Abstract. Blood inorganic phosphate, pyruvate and lactate have been determined during an intravenous glucose tolerance test in group of subjects with ischemic cardiovascular disease (ID) and in a group with overt diabetes mellitus. Among the subjects with ID, fasting blood inorganic phosphate decreased with decreasing glucose tolerance. Fasting levels of both pyruvate and lactate increased with increasing fasting blood glucose. None of these correlations was observed in the diabetics. After intravenous administration of glucose fall of inorganic phosphate was observed, the magnitude of which correlated positively to the glucose tolerance in all subjects. Blood pyruvate and lactate rose in the subjects with ischemic disease and normal glucose tolerance, and decreased in the diabetics after glucose injection. Positive correlations were observed in all subjects between the changes in pyruvate and lactate and the glucose tolerance.

Decreased glucose tolerance in the absence of clinical manifestations of diabetes mellitus occurs frequently among patients with ischemic cardiovascular disease (ID) (5 8, 21 22, 23 24). The metabolic aberration behind this decreased glucose tolerance is unknown. In the present investigation blood inorganic phosphate, pyruvate and lactate were determined during an intravenous glucose tolerance test in patients with ID and in patients with overt diabetes mellitus in an attempt to elucidate this problem.

The fall in blood inorganic phosphate after the administration of glucose has become an accepted indicator of peripheral glucose utilization. In 1934 Pollack et al. (16) demonstrated that the fall in inorganic phosphate after a glucose load in dogs was unaffected by hepatectomy and that there was no such fall in a visceral preparation with the liver present. Further evidence was presented by Nichols (15), who showed that no extrahepatic phosphate was added to dog liver during glycogen deposition, and by Gordon et al. (11), who showed that there was no net splanchnic uptake of phosphate after a glucose load in normal subjects. On this basis the fall in inorganic phosphate during an intravenous glucose tolerance test has been used to differentiate between the decreased glucose tolerance of liver disease—with normal peripheral glucose utilization—and that of diabetes mellitus (9).

Oral administration of glucose to normal subjects is followed by a temporary rise in blood pyruvate (12). This pyruvate response is diminished or absent in juvenile diabetics (1 7 20), while it is normal (1 20) or possibly delayed (7 12) in maturity onset diabetes. The changes in blood lactate after glucose administration have been studied much less but seem to follow closely the changes in pyruvate (2, 12).

MATERIAL

In the patients with ischemic cardiovascular disease this was manifested by myocardial infarction, angina pectoris and/or intermittent claudication. None of them had history of diabetes or glucosuria. Overt diabetes mellitus was defined as repeated observations of fasting blood glucose above 110 mg per 100 ml and glucosuria. All diabetics studied were of the maturity onset type. They had received dietary treatment only with the exception of three patients (2 in the phosphate group, and 1 in the pyruvate-lactate group), who had received oral sulphonylurea treatment. This was discontinued 36 hours before the test.

All subjects were in good physical and nutritional condition at the time of the test. With the exception of the diabetics none had evidence of diseases known to affect carbohydrate metabolism.

glucose half-life and fasting blood pyruvate ($R = 0.38$ $p < 0.05$) The lactate/pyruvate ratio on the other hand, did not correlate to fasting blood glucose, but decreased with increasing glucose half-life ($R = -0.38$, $p < 0.05$). The fasting levels of lactate and pyruvate in the diabetics did not fit into any of these correlations and did not differ from any of the ID groups. The lactate/pyruvate ratio in the diabetics was, however significantly greater than in the ID group with diabetic glucose tolerance.

The changes in lactate and pyruvate during the IVGTT are illustrated in Fig. 2. In the patients with ID both lactate and pyruvate rose to reach a maximum after 30 to 45 min. This rise was statistically significant in the normal and borderline groups for lactate, and in the normal group for pyruvate. In the diabetics both lactate and pyruvate fell after glucose administration, the decrease at 15 min being statistically significant. Negative correlations were observed in the total ID group between the glucose half-life and the changes in lactate and pyruvate at 15 min ($R = -0.42$, $p < 0.05$ and -0.57 $p < 0.01$ respectively) and at 30 min ($R = -0.46$ and -0.42, $p < 0.05$). The diabetics fitted well into these correlations and for the total material at 15 min $R = -0.69$ ($p < 0.001$) for both lactate and pyruvate, at 30 min $R = -0.67$ ($p < 0.001$) for lactate and $R = -0.57$ ($p < 0.001$) for pyruvate. No significant changes in the lactate/pyruvate ratio occurred after glucose administration.

DISCUSSION

In the ID group the fasting blood inorganic phosphate decreased with decreasing glucose tolerance, while the diabetics had intermediate fasting blood inorganic phosphate values despite their low glucose tolerance. Normal fasting blood inorganic phosphate in diabetes has earlier been reported by Forsham and Thorn (9) After glucose administration, the inorganic phosphate fell in all subjects, the fall being significantly smaller in the diabetics. This is in agreement with earlier observations (4 9 19). The fall of inorganic phosphate in the diabetics was smaller than that in the ID group with diabetic IVGTT This difference is probably explained by the fact that the glucose tolerance in these two groups also differed significantly (Table I), since negative correlations between glucose half-life and the decrease in phosphate observed after 30 45 and 60 min when all subjects were combined.

In the ID group, fasting blood lactate and pyruvate increased with increasing fasting blood glucose, while the lactate/pyruvate ratio decreased with decreasing glucose tolerance The diabetics deviated from this pattern—their lactate and pyruvate levels did not differ from any of the ID groups despite their higher fasting blood glucose, and they had significantly higher lactate/pyruvate ratios than the ID group with diabetic glucose tolerance.

The changes in blood pyruvate after a glucose load are determined by the balance between its rate of production from glucose and its rate of metabolism. Oral administration of glucose to maturity onset diabetics is followed by a normal pyruvate rise (1 7 12, 20), indicating that the decreased glucose utilization is balanced by a decreased pyruvate metabolism (14). In the present study using intravenous administration of glucose, only the ID patients with normal glucose tolerance showed a significant increase in blood pyruvate. With decreasing glucose tolerance the response in blood pyruvate diminished, and in the diabetics there was a significant fall in blood pyruvate 15 min after glucose administration. This lack of agreement with earlier observations is probably due to the different routes of glucose administration. Similar results were obtained by Smith (18) who studied the response of blood pyruvate 30 to 50 min after intravenous glucose injection in non-diabetic elderly subjects with normal and decreased glucose tolerance and in diabetics, and found insignificant changes in the diabetics in contrast to significant increases in blood pyruvate in the non-diabetic groups. There are several reasons why the response of blood pyruvate could be dependent on the route of glucose administration. Oral glucose tolerance tests generally use 50 to 100 g of glucose in contrast to 25 g in the present study Despite this lower dose the hyperglycemia is considerably higher after the rapid intravenous injection. Furthermore, after an oral glucose load a large fraction of the glucose dose is taken up by the liver directly in the portal circulation and never reaches the peripheral tissues (17).

The fall in blood pyruvate in the diabetics implies an increase in pyruvate utilization elicited

by the administration of glucose. Insulin release is probably not responsible for this effect since Moorhouse (14) has shown that insulin administration does not affect the pyruvate tolerance test in diabetics. Free fatty acids and ketone bodies are known to inhibit pyruvate oxidation in vitro in rat heart and diaphragm muscle (10), and the fall of these metabolites after glucose administration might be the cause of the apparent stimulation of pyruvate utilization.

The low glucose tolerance associated with ID differs from that of diabetes mellitus in that some mechanism prevents the patients from developing fasting hyperglycemia. According to the present results this metabolic condition is also associated with low fasting levels of inorganic phosphate and high fasting levels of pyruvate with a low lactate/pyruvate ratio. These changes were not observed in the overt diabetics. After an intravenous glucose load, on the other hand, there was no sharp delineation between the ID groups and the diabetics. In all subjects the glucose tolerance correlated to the changes in inorganic phosphate, lactate and pyruvate after glucose administration.

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THE POSTURAL PLASMA RENIN RESPONSE IN RENOVASCULAR HYPERTENSION

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Abstract. Plasma renin activity (PRA) in the supine position has been measured in 20 patients with signs of renovascular hypertension. Aldosterone secretion rate (ASR) was measured in 11 of these patients, while postural experiments were performed in 16. Operation, mostly reconstruction of the renal artery, was performed in 16 patients. PRA in the supine position was on average significantly higher in the patients than in normal subjects, although the range of values in the two groups overlapped. Postural experiments demonstrated qualitative as well as quantitative deviations from normal. When the increase in colloid osmotic pressure (ΔCOP) was plotted against the increase in PRA (ΔPRA), highly exaggerated PRA response was detected in four patients. The remaining patients formed population with significant linear correlation between ΔCOP and ΔPRA ($r = 0.54$, $p < 0.01$). The slope of the regression line did not differ significantly from the slope of the corresponding line, obtained from postural experiments in normal subjects. Hence, the group of patients with renovascular hypertension had either an exaggerated or normal postural PRA response. In the majority of patients PRA had increased already after 5 min in the standing position, in contrast to the findings in normal subjects. In half of the patients PRA levels reached plateau in the standing position, while in the other half peak value was attained on standing. Eleven patients were cured or improved by surgery. No correlation was found between surgical success and any of the different postural reaction patterns. The paired values for PRA and ASR fitted in with the general correlation between these values in normal subjects and some other hypertensive states.

In postural experiments on normal subjects and on patients with essential hypertension, Nielsen and Møller (13) found a significant linear correlation between the increase in hemoconcentration, as expressed by the increase in colloid osmotic pressure (COP), and the increase in plasma renin activity (PRA). The correlation line found for these values was less steep in patients with essential hypertension than in normal subjects, suggesting that renin secretion or release responded subnormally to postural changes in patients with essential hypertension.

In recent years it has become apparent that a normal PRA may be found in the presence of significant renal artery stenosis (4, 5, 7, 11). In order to improve the value of PRA determinations in the differential diagnosis of hypertension, we have examined the effect of postural changes on COP and PRA in patients with renovascular hypertension.

MATERIAL

Patients with renovascular hypertension

Twenty patients aged 22–60 years, 12 males and 8 females (Table I). All patients had fixed diastolic hypertension and uni- or bilateral renal artery stenosis, demonstrated by renal arteriography. The functional significance of the stenosis was demonstrated by urea-infusion urography and in most cases also by radio-hippuran renography. An exception was case 51, in which arteriography demonstrated left renal artery stenosis with numerous extrarenal decalcifications of the arterial tree. Abdominal aortography indicated similar changes in the intrarenal vessels. The urea-infusion urography was normal. Yet it was concluded that this patient suffered from disseminated arterial disease involving the kidney vessels and thereby causing the hypertension. For details concerning the patients, see Table I. Blood pressure before operation is indicated as the average blood pressure in the supine position for seven days before measurement of PRA and aldosterone secretion rate (ASR); blood pressure after operation is indicated as the average blood pressure in the supine position of several readings obtained in the Out-patient Clinic or during short admissions. Normotension is defined by diastolic blood pressure of 90 mmHg. Improvement after operation is defined by decrease in diastolic blood pressure of 20 mmHg or more.

Normal subjects

Thirty-three subjects, aged 16–67 years, mean age 40 years, 25 males and 8 females. All were healthy hospital

Table I. *Patients with renovascular hypertension*

FH = fundus hypertonicus. PRA = plasma renin activity. ASR = aldosterone secretion rate

Case no.	Sex	Age	Type	BP before operation	FH	Serum-creatinine	Supine PRA	ASR	Plasma Na k.	Operation	Result of operation (observation time indicated)
6	♂	56	Sten. a. ren. dx.	200/120	II	1.3	78	337	140 4.5	Not operated	—
8	♂	54	Sten. a. ren. sin.	250/140	IV	1.2	222	326	127 3.3	Endarterectomy of renal artery + patch graft	Normotensive, 20 mo. obs.: BP 110/75 FH = II
13	♂	41	Sten. aa. ren. bilat.	240/170	III	1.4	129	—	137 3.5	Transplantation of saph. magna to left renal artery. Right nephrectomy	Improved. 27 mo. obs. BP 150/100, FH I
19	♂	44	Sten. a. ren. sin.	210/130	III	0.7	37	—	142 3.6	Endarterectomy of renal artery + patch graft	Improved 16 mo. obs.: BP 150/105 FH II
28	♀	49	Sten. a. ren. dx.	200/120	II	0.8	26	200	140 3.2	Not operated	—
29	♂	54	Sten. ren. sin.	185/115	III	1.3	109	390	141 3.6	Transplantation of v. saph. magna to left renal artery	Normotensive 20 mo. obs. BP 115/85 FH I
34	♂	53	Sten. a. ren. dx.	200/130	IV	1.0	51	248	141 3.8	Transplantation of saph. magna to right renal art. Restenosis bilat. Left endarterectomy and right patch graft	Improved. 1 mo. obs. BP 140/100
39	♂	54	Sten. a. ren. dx.	200/95	0	—	4	—	139 3.4	Not operated	—
44	♂	60	Sten. aa. ren. bilat.	210/140	III	1.0	228	—	141 3.5	Transplantation of saph. magna to right renal artery. Left nephrectomy	Normotensive. 12 mo. obs. BP 145/90, FH II
48	♂	49	Sten. a. ren. dx.	200/140	II	0.9	123	—	140 3.2	Transplantation of saph. magna to right ren. art. Restenosis demonstrated	Not improved 12 mo. obs.
51	♂	45	Sten. a. ren. sin.	195/100	II	1.1	35	369	140 3.6	Not operated	—
69	♀	57	Sten. aa. ren. bilat.	190/110	I	0.9	21	—	—	Left endarterectomy transplantation of v. saph. magna to right ren. artery	Died postoperatively
76	♀	58	Sten. ren. sin.	220/120	II	0.9	26	242	140 3.4	Transplantation of saph. magna to left ren. artery	Not improved. 8 mo. obs.
84	♀	52	Sten. aa. ren. bilat.	200/120	II	1.6	27	180	141 3.6	Bilateral endarterectomy. Restenosis demonstrated	Not improved. 10 mo. obs.
91	♂	23	Sten. a. ren. sin.	220/150	I	1.0	37	—	140 3.5	Left nephrectomy	Normotensive 1½ mo. obs.

T ble I (*continued*)

Case no.	Sex	Age	Type	BP before operation	FH	Serum-crea-tinine	Supine PRA	ASR	Plasma Na+ K	Operation	Result of operation (observation time indicated)
92	♂		Sten. aa. ren. bilat.	200/130	III	1.3	32	—	141 3 8	Left endarterectomy + patch graft. Transplantation of saph. magna to right renal artery	Normotensive. 9 mo. obs. BP 115/80, FH = I
96	♂	44	Occlu-sion of right ren. art.	240/110	III	1.6	16	226	142 3.6	Right nephrectomy	Not improved. 7 mo. obs.
98	♂	46	Sten. aa. ren. bilat.	250/150	III	1.0	113	478	138 3.9	Bilateral endarter ectomy + patch graft. Four months later left nephrec tomy	Normotensive. 1 mo. ba BP 140/90
99	♂	48	Sten. a. ren. dx.	190/115	0	1 0	49	293	139 4.0	Transplantation of saph. magna to right renal artery	Improved. 6 mo. obs. BP 160/100
101	♂	40	Sten. ren. sin.	180/120	II	1.1	34	—	142 3.2	Left endarterec tomy	Normotensive. 7 mo. bs.

employees or patients admitted for minor conditions, and without cardiovascular or renal disease.

METHODS

Plasma renin activity (PRA) was measured by the method of Boucher et al. (1), slightly modified by Nielsen and Møller (12) (coefficient of variation ±12%). The results are expressed as ng angiotensin/10 ml of plasma/4 h of incubation.

The colloid osmotic pressure of plasma was measured in an electronic osmometer for quick, direct measurement of small samples, as described by Hansen (8). The results are expressed in cm H_2O. 95% confidence limits: ±0.5 mmHg. Standing plasma volume as % of supine value is computed as COP supine 100/COP standing.

Measurement of aldosterone secretion rate (ASR) was performed by the double isotope derivative method of Kliman and Peterson (10) (coefficient of variation ±7.7%).

The blood samples were obtained through an indwelling needle, placed in an antecubital vein, coagulation in the needle was prevented by flushing it with small amounts of 3.8% sodium citrate. Immediately before blood samples were obtained, 3 ml of blood were drawn and discarded to avoid contamination with sodium citrate from the needle. The blood samples were drawn in 20 ml disposable syringes. For each sample 18 ml of blood were transferred to siliconized Erlenmeyer flask immersed in ice-water and containing 2 ml of 3.8% sodium citrate. The remaining 2 ml of blood were used for determination of colloid osmotic pressure. Within one hour the blood in the Erlenmeyer flask was centrifuged at 0°C and 3 000 rpm for 15 min. The plasma was separated and kept at −20°C until renin activity was determined. In connection with the blood sampling approximately 10 μC H³-aldosterone was injected intravenously. The 24 h urine production from 7 m. to 7 a.m. on the following day was collected and ASR determined as described previously (14).

The normal controls received no medical treatment prior to determination of PRA. The hypertensive patients received no medication for at least seven days before the experiment. All subjects were on free salt intake.

All blood samples were obtained between 8 and 11 a.m. After at least 45 min of recumbency the blood samples for the determination of PRA and COP in the supine position were obtained. Plasma N and K concentrations were determined on the same sample. In 16 patients (Table II) postural experiments wer performed. The patients were seated to the standing position and allowed occasional steps. Blood samples for determination of PRA and COP were drawn at the intervals indicated in Table II. Aldosterone secretion rate was measured in 11 patients (Table I).

In normal controls PRA in the supine position was on average 14 ng/10 ml plasma 4 h incubation ±2 (S.E.M.), range 0–34 ng. In postural experiments on normal subjects Nielsen and Møller (12) observed that the values for PRA and COP reached plateau after 20 min in the standing position. In no case could an increase in PRA be demonstrated after only 5 min in the standing position. The same study revealed significant linear correlation between ΔCOP and ΔPRA from supine to plateau values. For postural experiments in 22 normal

Table II. *Postural experiments*

COP = plasma colloid osmotic pressure. PRA = plasma renin activity *Indices*: 0 sample after 45 min in supine position, 5 10, 20 and 30 = min after assumption of standing position

Case no.	PRA_0 COP	PRA COP	PRA_{10} COP_{10}	PRA_{20} COP_{20}	PRA_{30} COP_{30}	Standing plasma vol. in % of supine
6	78	235	140	120	92	84 7
	35 4	39.6	40.5	41.0	42.5	
8	222	650	700	735	—	75 7
	25.8	28.3	32.1	33.8	—	
13	129	262	234	223	252	94.3
	45.7	49.6	49.3	48.8	48.1	
19	37	39	33	41	—	89 0
	34 3	35.0	37.0	38.6	—	
28	26	26	40	27	—	87.6
	33.7	34.3	37.3	38.5	—	
29	109	159	132	257	—	88.6
	37.2	41.2	41 7	42.0	—	
34	51	67	97	92	72	81.9
	37.5	43.3	46.8	46..	45.4	
39	4	16	—	36	36	84 1
	33.2	37 1	—	39 1	39.8	
44	228	—	200	380	275	95.4
	50.3	—	51.5	52.6	53.9	
48	123	133	127	148	148	82 0
	34.7	38.5	40.1	40.3	44.2	
51	35	—	—	51	53	83.8
	25.9	—	—	30.6	31 1	
69	21	32	36	40	21	87.0
	26.8	28.6	30.5	31.0	30.6	
76	29	40	28	43	43	88.2
	36.7	38.8	40.7	41.0	42.2	
84	31	—	29	40	40	84.2
	31 4	—	34 7	37 6	37.0	
91	37	—	—	47	48	88.0
	41.8	—	—	48.0	47.0	
92	37	—	—	55	37	88.2
	36.4	—	—	42 0	40.5	
Mean						86.4 ±1.2 S.E.M.

subjects the regression line was $y = 2.8 + 1.0$, $r = 0.85$ $p < 0.001$ (13). The aldosterone secretion rate in 14 normal subjects was on average 97 μg/24 h ± 15 (S.E.M.).

RESULTS

PRA in the supine position

In patients with renovascular hypertension the mean PRA was 70 ± 15 ng (S.E.M.). This is significantly higher than in normal controls ($p < 0.001$). No correlation was found between PRA in the supine position and blood pressure or eyeground changes (Table I) or between PRA in the supine position and the postoperative result in the 15 patients who underwent surgery (Table III).

Postural experiments

All patients demonstrated a normal postural increase in COP A plateau was attained in 20 min. Plasma volume in the standing position, expressed

as percentage of the value in the supine position, was not significantly different from normal values (normal mean standing plasma volume as percentage of the value in the supine position 84 6 $\pm$ 1.0 (13)) ($0.20 < p < 0.25$).

In all patients investigated, except in case 19 PRA increased significantly following a change of posture (the increase exceeded the 95 % confidence limit of the method in at least one standing value). Eight patients attained plateau values for PRA (cases 8, 13 39 48 51 76, 84 and 91), while PRA in six patients reached a peak value and then declined (cases 6, 28 34 44 69 and 92). In 8 of 11 patients investigated, PRA had already increased significantly after 5 min in the standing position (cases 6, 8, 13 29 34 39 69 and 76).

The success of operation was not related especially to any of these patterns of response.

Fig. 1 demonstrates the correlation between ΔCOP and ΔPRA from supine to plateau values, or to values attained after 20 and 30 min in the standing position. From this figure it appears that seven pairs of values are situated far from the remainder These seven pairs of values with very high PRA originate from four patients (cases 8 13 29 and 44). The group of patients with the above mentioned exaggerated postural PRA response had a mean PRA in the supine position

Table III. *Plasma renin activity in supine position* (PRA_s) *in operated patients in relation to surgical results.* Case 69 *omitted (died of myocardial infarction a few days after operation)*

Case no.	PRA_s Normotensive	Improved	Not improved
8	222		
13		129	
19		37	
29	109		
34		51	
44	228		
48			123*
76			29
84			27*
91	37		
92	32		
96			16
98	113		
99		49	
101	34		

* Patients with restenosis.

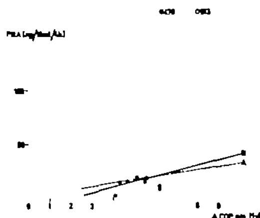


Fig. 1 Correlation between increase in plasma colloid osmotic pressure (ΔCOP) and increase in plasma renin activity (ΔPRA) from the supine to the standing position. Exaggerated postural response, ○ Normal postural response, ● Dashed line (*A*), regression line for normal subjects. Full line (*B*), regression line for patients with normal postural response.

of 172 $\pm$ 31 ng angiotensin/10 ml plasma/4 h incubation, while the remaining patients had a mean PRA in the supine position of 4 $\pm$9 ng. The difference is significant ($p < 0.001$). (Fig. 2). The success of operation did not seem to be especially related to either a high PRA in the supine position or to the exaggerated PRA response to posture.

The correlation ΔCOP–ΔPRA for patients with a normal PRA response to posture is demonstrated in Fig. 3 The correlation is linear and significant ($= 0.56$, $p < 0.01$). The regression line is $y = 4.6x - 9.3$ In Fig. 3 is indicated the regression line for postural experiments in normal subjects. The slopes of the two lines are not significantly different ($0.10 < p < 0.20$).

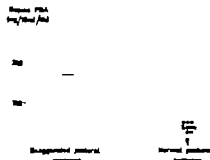


Fig. 2 Plasma renin activity in the supine position (PRA) in relation to the character of the postural response.



Fig. 3 The correlation between increase in plasma colloid osmotic pressure (ΔCOP) and increase in plasma renin activity (ΔPRA) from supine to standing position in patients with normal postural response. Regression line for normal subjects, *A*. The 95% confidence limits ($\pm 2 \quad S_m$) for this line shown as dashed lines. Regression line for the patients, *B*

Aldosterone secretion rate

Mean ASR for the 11 patients investigated was 229 ± 27 μg/24 h (S.E.M.). This is significantly higher than in normal subjects ($p < 0.001$). Fig. 4 depicts the correlation between PRA and ASR in these patients. The figure shows values for normal subjects, patients with essential hypertension and patients with renal parenchymatous disease and hypertension. It is readily seen that the values for patients with the renovascular hypertension participate in the general good correlation.

DISCUSSION

This investigation has demonstrated that the mean PRA in patients with renovascular hypertension examined in the supine position is significantly higher than in normal subjects, although the range of PRA values in both groups is wide and overlapping. Similar results have previously been found by several investigators (2, 6, 16). It is also apparent as found by others (4 5 7 11), that a normal PRA in the supine position does not exclude the presence of significant renal artery stenosis.

The response of PRA to posture in patients with renovascular hypertension differs qualitatively as well as quantitatively from the response in normal subjects. In the majority of patients an increase in PRA was found already after 5 min in the standing position this is not seen in normal subjects (12). About one half of the patients demonstrated a peak value of PRA, while the rest of the patients attained a PRA plateau as in normal subjects (12). In four of the patients an exaggerated increase in PRA was found. Mean PRA in the supine position was significantly higher in this group of patients than in the remainder No correlation between surgical success and any of these patterns of response was found.

Nielsen and Møller (13) found a significant correlation between ΔCOP and ΔPRA in pa-

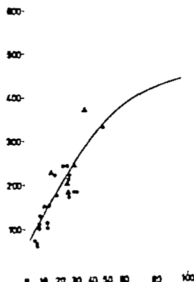


Fig. 4 The correlation between plasma renin activity (PRA) and aldosterone secretion rate (ASR) measured in the same 4 hours. Normal subjects, patients with essential hypertension and with renal parenchymatous hypertension, ● Patient with renovascular hypertension, ▲

tients with *essential* hypertension and fundal changes of grades I–III. The slope of the line was significantly less than that for normal subjects, indicating a decreased response of PRA to postural changes in these patients. In patients with *renovascular* hypertension and a normal PRA response to posture, a significant linear correlation between ΔCOP and ΔPRA was also found. The slope of the regression line did not deviate from the normal, but the *r* value was low in contrast to the *r* value for normal subjects (13), presumably because many patients did not reach a stable PRA value in the standing position. It may be concluded, therefore, that patients with renovascular hypertension are either hyperresponsive or normoresponsive to the postural stimulus, whereas patients with essential hypertension are hyporesponsive. In a given patient, however this difference is often too small to be of diagnostic value.

Postural experiments in renovascular hypertension have previously been performed by Cohen et al. (4) and Weidmann et al. (19). In five patients with a normal PRA in the supine position, Cohen et al. (4) found that the standing position induced a rise in PRA to abnormally high values. In the present work it was found that the four patients with an exaggerated response of PRA to posture had grossly elevated PRA values in the supine position. In four patients with renovascular hypertension, Weidmann et al. (19) found an exaggerated response of PRA to posture, when this stimulus was combined with salt depletion. In the present work salt depletion was not carried out.

In normal subjects renal blood flow decreases considerably upon rising from the supine position (3 17). It is likely that posturally induced alterations in pressure and flow in a kidney with stenotic artery may differ both qualitatively and quantitatively from those occurring under normal circumstances. This might explain the abnormal stimulation of renin, seen in some patients (for a review of mechanisms of renin stimulation, see Page & McCubbin (15)). Kaneko et al. (9) found that a decrease in systemic blood pressure, induced by injection of sodium nitroprusside, was followed by an increase in renal vein renin concentration in normal subjects and in patients with renovascular hypertension. The threshold blood pressure, below which the effect occurred, was higher in patients with renovascular hypertension than in normal subjects. These findings indicate that the renin stimulation mechanism in renovascular hypertension is more sensitive than in normal subjects, and are compatible with the findings in the present work as well as with those of Cohen et al. (4) and Weidmann et al. (19).

The aldosterone secretion rate was in the normal range in two patients and clearly elevated in the remaining nine patients investigated. It appears from Fig. 4 that the values for PRA and ASR found in patients with renovascular hypertension participate in the general correlation between PRA and ASR in normal subjects and patients with hypertension of other types, excluding primary hyperaldosteronism. Streeten et al. (18) have reported similar findings. This is evidence of the physiological significance of the PRA measurements. In addition, the higher than normal values for PRA in the supine position in patients with renovascular hypertension, as well as the exaggerated response of PRA to postural changes, observed at least in some of these patients, suggest a role for the renin system in renovascular hypertension.

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LIVER CIRRHOSIS IN THREE SCANDINAVIAN COMMUNITIES

An Attempt at a Critical Evaluation of Available Epidemiologic Data

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Abstract. Epidemiologic data on liver cirrhosis during the period 1951–1968 have been studied in one Danish and two Swedish communities. During the period of observation the liquor consumption in Denmark has shown a slow continuous increase. In Sweden there has been an increase of similar size but most of it occurred between 1955 and 1957 probably due to a drastic liberalization of liquor legislation. In one of the Swedish materials—essentially urban—there was a small but distinct increase in morbidity and mortality of liver cirrhosis within the years immediately following the alcohol let-loose. This increase was especially marked in middle-aged males from social group II. On the whole there is probably a slight increase in the incidence of cirrhosis throughout the period. In the Swedish rural region a slight tendency to increased incidence of liver cirrhosis similar to that observed in the urban region was noted in the years following 1955. In total, there is a considerable increase in registered morbidity and mortality in the region during the whole period. This increase clearly parallels the increased facilities for hospital and specialist care in the region. In the Danish material there is a marked increase in the incidence of liver cirrhosis also starting in the years after 1955 and then continuing throughout the period. In contrast to the Swedish series the increased incidence in the Danish material is most marked among females, especially elderly females. Epidemiologic data on liver cirrhosis have often been used as a measure of the extent of alcoholic abuse. Reasonably they may give some information in short-time studies on well-defined populations, especially in the case of sudden sharp changes in drinking habits. In longitudinal studies on a more vaguely defined population, and especially in the comparison between different populations, any attempt to estimate alcoholic abuse based on such data seems extremely hazardous.

It is generally accepted that cirrhosis of the liver is more common among alcohol addicts than in the population as a whole. It has even been suggested that epidemiologic data on liver cirrhosis might be used for measuring the extent of alcoholic abuse. Such a procedure has in fact been recommended by the WHO Alcohol Subcommittee Study Group (22). The basis of underlying assumptions for this procedure seems not altogether well-founded. The quantitative correlation between alcohol consumption and the incidence of liver cirrhosis probably differs greatly between different types of populations. This immediately limits the use of this method to longitudinal studies. Furthermore, the representativity of the epidemiologic data available has been insufficiently studied. The present study is an attempt at a critical evaluation of various epidemiologic parameters.

There are undoubtedly some observations pointing to a close correlation between the mortality in liver cirrhosis and the average alcohol consumption under certain, rather exceptional circumstances, e.g. in connection with World Wars I and II (1, 14, 19, 20, 21). Analogous observations in peace time are more scarce (6, 15).

Until 1955 a state-controlled liquor distribution system had existed in Sweden for about 40 years, allowing a maximum purchase of 1–4 l of hard liquor a month per person. Alcohol consumption in restaurants was permitted only together with meals, and there were no public bars. On Oct. 1, 1955 the restrictions were abolished. This gave an unique opportunity to study the effect of an unrestricted liquor supply. The total consumption of hard liquor increased from 45 mill. l in 1953 to 58 mill. l in 1956. A considerable increase in the morbidity and mortality of liver cirrhosis was also reported in two Swedish cities (Malmö and Stockholm) for the years following 1955 (2, 3, 4). The present study presents some epidemiologic data on liver cirrhosis in one urban and

semi-urban and one more rural Swedish community For comparison a corresponding study has been made in a Danish community the city of Arhus.

MATERIAL AND METHODS

The communities and their medical facilities

The County of Uppsala (C Region) had population of 155 000 in 1951 increasing to 193 500 in 1968 F rty seven per cent of the population was urban in 1951 and 60% in 1967 The University Hospital is the principal hospital of the region, with 180 medical beds and 250 surgical beds. There are also tw smaller hospitals, with 75 and 80 beds, respectively

The University Hospital is served by Pathology Department, where practically all patients dying in the hospital are autopsied. In the 1960s the Department also served mental hospital, home for the aged and some *hospitals for chronic disorders located in the town.*

The County of Dalecarlia (Kopparbergs län, W Region) had population of 267 000 in 1951 and of 283 000 in 1965. Seventy-six per cent of the population was rural in 1951, 68% in 1964. Until 1955 the Central Hospital of Falun possessed the only clinic for internal medicine in the region. I 1955 1959 and 1963 medical clinics were established at three smaller hospitals in the region, increasing the total number of beds designed for internal medicine from 105 in 1951 to 290 in 1964

There was no department of pathology in the region until 1964. Prior to that year autopsies were performed on selected cases only and usually not by trained pathologist.

The City f Arhus had population of 116 000 in 1951 and of 118 000 in 1965 The city has tw hospitals, with three medical departments for the urban residents. The number of beds for internal medicine was 326 in 1965 The Department of Pathology serves the hospitals mentioned, as well as home for the aged.

Clinical series

Only patients over 20 years of age are included and registered as to the year in which the diagnosis was originally made.

C Region. The records of all patients registered under the diagnosis of cirrhosis of the liver or chronic hepatitis in the departments of internal medicine, surgery and infectious diseases were examined.

W Region. Th. records of all patients from the medical clinics registered under the abov diagnoses were collected. In the surgical clinics few cases of cirrhosis were bo registered. However the records did not usually permit proper evaluation of the diagnoses, nor were the principles of registering the secondary diagnoses consistent. This was also the case ith the mixed medical-surgical wards existing before the establishment of departments of internal medicine in the smaller hospitals. For that reason the present study was confined to the cases found in the internal medical wards.

Arhus. The records f all patients from the medical departments registered under the above diagnoses were examined. For similar reasons as in W Region the records from other departments could not be used.

Only permanent residents of the regions were included. The diagnostic criteria required were as follows

1 Histological diagnosis at autopsy or biopsy

2. Typical clinical signs and symptoms of portal hypertension.

3. Laboratory signs f chronic liver insufficiency and hepatocellular damage including a typical serum protein pattern. The deranged biochemical tests had to be observed repeatedly during period of one year or more.

Autopsy series (over 20 years of age)

Data were collected from the autopsy registers in the departments of pathology in Uppsala and Arhus. The Uppsala register contains the diagnoses of clinical significance found at autopsy Diagnoses of minor importance are often omitted.

The Arhus register contains complete list of all diagnoses found at autopsy

Officially reported mortality in liver cirrhosis (all ages)

C and W Regions. These figures are based on the number of death certificates in which liver cirrhosis was stated as being the immediate or underlying cause of death. In order to test the degree f correspondence between the officially reported cases and the clinical and autopsy series, the death certificates were individually examined. The certificates from 1952 1960 for C Region and from 1951 1960 for W Region ere obtained from the N tional Central Bureau of Statistics. No other certificates from the *period of study ere available. This scrutiny also provided* facilities for critical evaluation of the official reports.

Series f specific mortality (over 20 years of age)

This series includes all patients with the diagnosis of liver cirrhosis dying from pper gastrointestinal bleeding and or hepatic coma. When liver cirrhosis is complicated by peptic ulcer it may be difficult to determine the exact site of the fatal bleeding. For that reason cases have been included in uch varicose veins ere not actually demonstrated. In C and W Regions information on specific mortality has been collected from all the materials under study—clinical records, death certificates and autopsy registers (C Region).

Some patients died at hospitals or clinics not included in the clinical study or at home after leaving the hospital where the diagnosis was made. A few were not autopsied although they died in hospital. These circumstances explain the pparent discrepancy between some of the figures in the series of pecific mortality and the clinical or autopsy series.

I Arhus, information was collected from clinical records and from clinical statements on the autopsy requisition.

Malignant lymphoma (over 20 years of ge)

In C Region all patients registered under the diagnosis of malignant lymphoma (WHO 200, 201 204) in the medical ards and at the Department of Radiotherapy ere included.

In W Region all patients registered under the same diagnoses were collected from the medical wards. No special department of radiotherapy exists in this region.

RESULTS

C Region

Clinical series

In the 18-year period of study 230 cases of liver cirrhosis were found which satisfy the criteria given above, 125 men and 105 women (male/female ratio = 1.2 1). The diagnosis was made or suspected intra vitam in 167 cases, 94 men and 73 women. In 63 cases (27%) the diagnosis was more or less unexpectedly found at autopsy The diagnosis was based on biopsy or autopsy in 78% of the cases, on clinical signs of portal hypertension in 16% and on deranged biochemical tests in 6% Eighty-two cases registered under the diagnoses were excluded from the study either because they did not fulfil the diagnostic criteria or because the diagnosis had originally been made before 1951. All but eight of the diagnoses discarded were made after 1955

The yearly incidence is demonstrated in Fig. 1 *a*. The fraction of cases diagnosed post mortem was not significantly altered during the period. The age specific incidence is shown in Fig. 4. Fig. 5 shows the changes in age distribution during the period of study Socio-economic groups for the male patients are presented in Figs. 6 and 7

Socio-economic group I includes employers and employees, *owners and heads of large or medium-sized* businesses (managers and acting managers etc.), and officials in comparatively responsible positions, as a rule with university education.

Socio-economic group II also includes both employers and employees in lower positions—clerical and sales staff, foremen and self-employed craftsmen. Most farm-owners and tenant farmers are placed in this group.

Socio-economic group III may be characterized as a worker's group—labourers, service workers, operatives and salaried craftsmen. Among the women, social information was lacking or fragmentary in one-third of the cases.

The majority of cases were classified as portal cirrhosis. Typical biliary cirrhosis was found in three men and five women, cardiac cirrhosis in one man and one woman. Ten patients of each sex had diabetes mellitus.

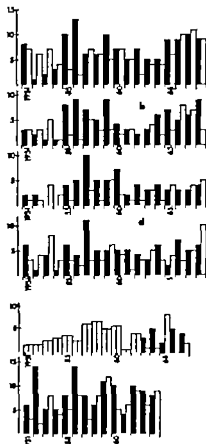


Fig. 1 C Region. (*a*) Yearly incidence of newly diagnosed cases of liver cirrhosis (number of cases) (*b*) Yearly incidence of newly diagnosed cases of liver cirrhosis in the Department of Medicine, Uppsala University Hospital. (*c*) Yearly specific mortality in liver cirrhosis (no of cases). (*d*) Yearly no. of cases with liver cirrhosis found at autopsy ( ) Officially reported mortality in liver cirrhosis (no. of cases). As there is no information with regard to the sex of the cases before 1963, each column from 1951 to 196_ represents half the number of cases for the year. (*f*) Yearly incidence of newly diagnosed cases of malignant lymphoma (no. of cases) Filled columns, males, open columns, females.

Regular daily consumption of strong liquor was recorded in about 60% of the males. Proven abuse was reported in at least half of this group (30%). Reports of proven or suspected alcoholism in women were recorded in a few instances only Sixty-three per cent of the cases diagnosed post mortem were 70 years or older Of the patients dying with previously diagnosed cirrhosis, 32% were 70 years or older at death. Thirty-seven per cent of the post mortem group and 45% of the intra vitam group belonged to socio-economic groups I–II.

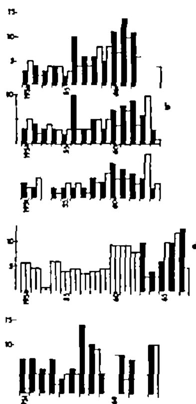


Fig. W Region. (a) Yearly incidence of newly diagnosed cases of liver cirrhosis (no. of cases). (b) Yearly incidence of newly diagnosed cases of liver cirrhosis in the Department of Medicine Falun Central Hospital. (c) Yearly specific mortality in liver cirrhosis (no. of cases). (d) Officially reported mortality in liver cirrhosis (no of cases, cf. Fig. 1). (e) Yearly incidence of newly diagnosed cases of malignant lymphoma (no. of cases). Symbols as in Fig. 1

The newly diagnosed cases from the Medical Department of the University Hospital are shown separately in Fig. 1 *b* (These cases are, of course, also included in Fig. 1 *a*)

Autopsy series

The cases of liver cirrhosis found at autopsy in the Department of Pathology of Uppsala University are presented in Fig. 1 *d*. Typical cases of biliary and cardiac cirrhosis are excluded. The total number of autopsies was 421 in 1951 711 in 1960 and 1 086 in 1968. The percentage of autopsied cases dying in hospitals other than the University Hospital increased from less than 2% in 1951 to 28% in 1968. In 30 cases the post mortem diagnosis of cirrhosis was not present among the diagnoses registered in the hospital chart and was not mentioned in the death certificate signed by the clinician. The reason for this was obviously that the cirrhosis was not judged to be the major cause of death.

Officially reported mortality

The officially reported mortality for C Region is shown in Fig. 1 *e* All certificates from the years 1952–1960 were based on diagnoses made in hospitals. Autopsy was done in 72% of the cases. In 16% the diagnosis was made without autopsy in wards for chronic disorders. Nineteen per cent (4/21 cases) of the certificates from 1952–1955 referred to patients who could not be found in either the clinical or the autopsy series. The corresponding figures for 1956–1960 were 37% (19/51 cases). Four of these missing patients were less than 20 years of age.

Specific mortality

The specific mortality is presented in Fig. 1 *c*. The series consists of 42 patients dying from bleeding in the gastro-intestinal tract and 63 patients dying from liver insufficiency

Malignant lymphoma

The newly diagnosed cases of malignant lymphoma are presented in Fig. 1 *f* age specific incidence in Fig. 8 and socio-economic grouping of the male patients in Figs. 6 and 7

W Region

Clinical series

During the period 1951–1964 184 cases were found in the medical departments, 97 men and 87 women, corresponding to a male/female ratio of 1 1 1 The diagnosis was made intra vitam in 143 cases, 80 men and 63 women. In 41 cases (2_%) the diagnosis was found at autopsy—all but four were found after 1957

The diagnosis was based on biopsy or autopsy in 70% on clinical signs of portal hypertension in 21% and on deranged biochemical tests in 9% Fifty-four cases (51 after 1956) registered under the diagnosis of cirrhosis did not satisfy the diagnostic criteria and were thus excluded. The yearly incidence of liver cirrhosis is seen in Fig. 2 a. Age specific incidence is seen in Fig. 4 and Fig. 5 shows the changes in age distribution for three consecutive periods of 4 ½ years. Figs.

6 and 7 show the socio-economic grouping of the male patients.

Portal cirrhosis was the dominant type of cirrhosis found. Typical biliary cirrhosis was found in one woman and cardiac cirrhosis in three men and three women. Regular daily consumption of hard liquor was reported in about 50% of the males. Alcoholism from the social point of view was noted in about 25%. Only a few instances of proven or suspected alcoholism in women were reported. Ten men and eight women had diabetes mellitus.

Specific mortality

The yearly specific mortality due to cirrhosis is demonstrated in Fig. 2 c. Liver insufficiency (hepatic coma) was found to be the cause of death in 57 patients, bleeding from the gastro-intestinal tract in 3 patients.

Officially reported mortality

The officially reported mortality from liver cirrhosis (based upon death certificates) is presented in Fig. 2 d. During the years 1951–1960 liver cirrhosis was stated to be the cause of death in 93 cases (47 men and 46 women). Autopsy was performed in 53 cases. In 11 cases the diagnosis was made outside hospital without autopsy. Thirty five patients died in medical wards. Twelve patients (1951–1955 six patients, 1956–1960 six patients) died in surgical wards or mixed medical-surgical wards not included in the present study.

Malignant lymphoma was diagnosed in 122 men and 60 women. The yearly incidence of the medical departments is presented in Fig. _ c the age specific incidence in Fig. 8, and the socio-economic grouping of the male cases in Figs. 6 and 7.

Arhus

Clinical series

A clinical diagnosis of liver cirrhosis based on the above criteria was made in 103 male and 149 female patients (male/female ratio 1 1.45) in the three medical departments of the city of Arhus. In 202 cases (80%) the diagnosis was made intra vitam, in 50 cases (20%) cirrhosis was found more or less unexpectedly at autopsy. The diagnosis was based on biopsy or autopsy in 73% of the cases, in 15% on clinical signs of portal hypertension and in 12% on deranged biochemical tests. Eighty cases registered as liver cirrhosis

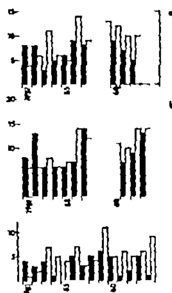


Fig. 3. Arhus. (a) Yearly incidence of newly diagnosed cases of liver cirrhosis (no. of cases). (b) Yearly number of cases with liver cirrhosis found at autopsy. (c) Yearly specific mortality in liver cirrhosis (no. of cases). Symbols as in Fig. 1.

during the period did not satisfy the diagnostic criteria and were excluded. So were 5 cases of typical biliary cirrhosis. Seventy per cent of the cases diagnosed at autopsy were 70 years or older compared with 57% of the patients who died with previously diagnosed liver cirrhosis. The social grouping was about the same in the two groups of patients. The yearly incidence of newly diagnosed cases is demonstrated in Fig. 3 a, the age specific incidence and social grouping in Figs. 4, 5, 6 and 7. Diabetes mellitus was reported in

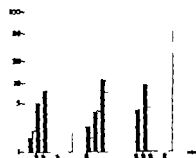


Fig. 4. Age specific morbidity of liver cirrhosis. Average annual incidence per 100 000 by 10-year age groups (semi-log. scale). (a) C Region. (b) W Region. (c) Arhus. Symbols as in Fig. 1.

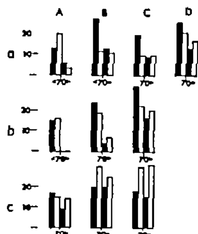


Fig 5 Trends of age distribution among cases with newly diagnosed liver cirrhosis. The columns illustrate the number of patients below and above 70 y for each period. (*A*) Jan. 1951 – June 1955 (*B*) July 1955 – Dec. 1959 (*C*) Jan 1960 June 1964 (*D*) July 1964 Dec. 1968. (*a*) C Region. (*b*) W Region. (*c*) Arhus. Symbols as in Fig. 1

20 men and 10 women. The clinical records in most cases contain no information on drinking habits.

Autopsy series

Liver cirrhosis (excluding biliary and cardiac cirrhosis) was found at autopsy in 144 men and 165 women (male/female ratio 1 1 15). The yearly incidence is presented in Fig 3 *b* From the autopsy series 222 cases were patients from the medical departments. In 60 of these 222 cases the post mortem diagnosis of cirrhosis was not recorded in the clinical charts. These 60 cases are

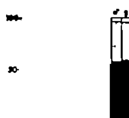


Fig 7 Percentual distribution of socio-economic groups among (*a*) C Region, male patients with liver cirrhosis. (*b*) C Region, male patients with malignant lymphoma. (*c*) W Region, male patients with liver cirrhosis. (*d*) W Region, male patients with malignant lymphoma. (*e*) Arhus, male and female patients with liver cirrhosis. Symbols as in Fig. 6.

thus not included in the clinical series. The total number of autopsies at the Department of Pathology increased during the period of study from 479 in 1951 to 1 091 in 1964 These numbers include cases of all ages and a few patients referred from other communities. The total number of deaths among the residents of Arhus was 984 in 1951 and 1 ‿85 in 1963

Specific mortality series

The yearly incidence of deaths due to either hepatic coma or bleeding into the gastro-intestinal tract is presented in Fig. 3 *c*

DISCUSSION

Clinical series

The ideal situation for an epidemiologic study exists when all cases within a population are diag-

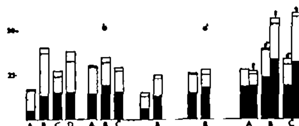


Fig 6 Socio-economic grouping of the clinical material during the periods: (*A*) Jan. 1951 June 1955 (*B*) July 1955 – Dec. 1959 (*C*) Jan. 1960 June 1964, (*D*) July 1964 Dec. 1968 The columns represent the number of cases. (*a*) C Region, male patients with liver cirrhosis. (*b*) C Region, male patients with malignant lymphoma. ( ) W Region, male patients with liver cirrhosis. (*d*) W Region, male patients with malignant lymphoma. (*e*) Arhus, male and female patients with liver cirrhosis. □ socio-economic group I ▢ socio-economic group II ■ socio-economic group III.

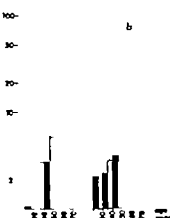


Fig 8 Age specific morbidity of malignant lymphoma. Average annual incidence per 100 000 by 10-year age groups (semilog. scale). (*a*) C Region. (*b*) W Region. Symbols as in Fig. 1

nosed and known soon after the development of the first symptoms. For most diseases this situa tion will probably never occur in a large community The diagnosis may not be made, it may be incorrect, or a correct diagnosis may be established but never officially reported. The difficulties are especially great in studies of a disease that has an unspecific and variable symptomatology such as liver cirrhosis. In the case of liver cirrhosis there is an additional difficulty in the fact that the diagnosis by definition is histological.

A clinical material of the type used in the present study does not, of course, represent the total morbidity Only a selected number of patients with liver cirrhosis will be admitted to hospital. We believe, however that the cases presented here represent the majority of all cases diagnosed intra vitam in the communities studied. Assuming this fraction to be reasonably constant the number of newly diagnosed cases from the medical wards would give a good relative measure of the morbidity This figure would be especially use ful in estimating incidence variations within a cer tain community However the fraction constitut ing our clinical material has presumably not been constant during the period of observation due to several factors.

The diagnostic facilities have improved, e.g. electrophoresis and percutaneous biopsy have been used much more extensively in recent years. This could be assumed to cause an increase in the incidence observed, not corresponding to an increase in actual morbidity The risk of such an error will be particularly great when histological examination is made a prerequisite for the diagnosis. In accordance with other authors (4 19) we have accepted a certain number of non-histologically diagnosed cases in spite of the fact that this car ries a certain risk of overdiagnosis. We believe the improved diagnostic facilities to be a minor source of error in the present investigation, since the percentage of unexpected cases found at autopsy has not decreased but has actually shown a slight increase.

Changes in the availability of hospital beds will also influence the number of diagnoses. So will the access to specialists in internal medicine. While the situation has been fairly constant in the C and Arhus Regions the situation in W Region has changed considerably The number of beds in departments of internal medicine in W Region has more than doubled during the period. The number of specialists has increased still more. The effect of this change on the diagnostic incidence is difficult to evaluate. In the present study an attempt to estimate this effect has been made by comparing the incidence of liver cirrhosis with that of malignant lymphoma from the same departments. The choice of malignant lymphoma was made for several reasons. The chances are, in all probability that the true mor bidity of this type of disease has been constant during the period. It affects approximately the same age groups as does liver cirrhosis (Figs. 4 and 8), and it should reasonably be unaffected by factors influencing the development of cir rhosis such as infectious hepatitis, alcoholic abuse and malnutrition. Changes in the observed incidence of malignant lymphoma should reasonably reflect the possibility of the patient obtaining a correct diagnosis, i.e. essentially the possibility of obtaining hospital or specialist care.

Another factor that may influence the incidence of a diagnosis is the attention that the doctor pays to this special disease. Undoubtedly in Sweden special interest has been focused on liver cirrhosis since the abolition of the liquor rationing system. There is no reason to assume a similar phenomenon in Denmark. The increasing per centage of registered diagnoses based on insuf ficient clinical data found in the Swedish series might be an indication of an increased consciousness of the disease. Thus we have found several instances where acute alcohol intoxication and a single elevated serum transaminase value were the sole basis for the diagnosis.

Another factor of importance is the number of autopsies performed, as more than 20% of the clinically reported diagnoses are made post mor tem. An increase in the number of autopsies, particularly among elderly patients, will thus tend to cause an increase in the number of diagnoses (1). While the situation has been fairly constant in the C and Arhus Regions, the number of autopsies has steadily increased in the W Region. When studying a disease with high death rate and short mean survival time after diagnosis, the yearly mortality should be a reasonably good measure of the morbidity Under ideal circumstances the curve of the yearly mortality should be similar to that of the morbidity with a phase lag corresponding to the mean su

the case of liver cirrhosis several authors (3 4 13 19 20) have reported high death rates during the first years after clinical diagnosis. There are reasons to believe that the mean survival time has increased moderately since 1951 owing to the introduction of more effective diuretics, more effective treatment of liver coma, etc. (13). The small number of cases in the present series does not permit an estimation of this assumed increase.

Three types of mortality data are used in the present study—autopsy data, death certificates and specific mortality"

The autopsy series includes all cases found at autopsy regardless of the cause of death. The number of cases found in per cent of the total number of autopsies should correspond to the term prevalence at death" proposed by Markush and Seigel (10, 16). Evaluation of figures obtained in this way is, however extremely difficult because of the difficulties in defining the population at risk when the autopsy rate is not fairly close to 100% In the C and Århus Regions the autopsy rate has been fairly constant at the medical centres contributing to the clinical series. The autopsy rate at homes for the aged and similar institutions shows a quite evident increase during the period of study in C Region, and a corresponding tendency may be seen in Århus. During the period of study the number of deaths occurring in hospitals providing regular post mortem examination has thus increased in relation to the total number of deaths. The added group most probably contains a selection of elderly and disabled people.

When cirrhosis is of minor clinical importance, e.g. in a case of diabetes mellitus with disseminated vascular lesions, a moderate degree of liver cirrhosis may be ranked as the last diagnosis of the 10 to 15 listed in the autopsy record. In Århus the mean number of diagnoses registered in each case of cirrhosis was 7 (range 3–11) in 1951 increasing to 12 (range 6–19) in 1964. In Uppsala (C Region) the number of diagnoses for each patient in the register utilized for the present study has shown a slight decrease during the period. The probability of an accessory diagnosis being registered at all will obviously increase with increasing total number of diagnoses registered.

The officially reported mortality in liver cirrhosis is based upon observations at autopsy in 60–75% of cases. It will consequently be influenced by the same errors as the autopsy series. In the remaining cases practically all certificates are based on clinical diagnoses made intra vitam in hospital. The diagnostic validity for this group will, of course, be influenced by the same sources of error as the clinical series. The number of deaths from cirrhosis certified without autopsy or hospital diagnosis is negligible.

The official mortality reports are based upon death certificates giving liver cirrhosis as the immediate or underlying cause of death. This, of course, means a severe quantitative limitation of the material in relation to the true incidence of cirrhosis. During the last five years the reports also contain information as to contributory causes of death The addition of all cases with liver cirrhosis registered as contributory" will approximately double the total number The choice of the underlying cause of death is often difficult. This is especially the case when the patient is suffering from several diseases. The principles by which the diagnoses are recorded vary from one physician to another and will strongly influence the official mortality figures.

In an attempt to estimate the size of the error introduced in this way we found that 20% of the diagnoses recorded at autopsy were not recorded clinically in C Region and in Århus. Usually the number of diagnoses on the death certificates will be identical with or less than those listed in the clinical chart. Consequently it seems safe to assume that less than 80% of the cases found at autopsy are officially reported.

Specific mortality is a function of the number of cases of advanced liver cirrhosis. The time between the onset of cirrhosis and the development of severe symptoms is variable, but usually extends over years or even decades (7 8 9 13). Changes in primary morbidity will thus influence very slowly the specific mortality rate. This rate will be much more sensitive to sudden changes in precipitating environmental factors or changes in therapeutic facilities. The symptoms of gastro-intestinal bleeding or hepatic coma are sufficiently dramatic to necessitate the patient s being taken to a hospital, and in practically all cases the correct diagnosis will be made or at least suspected. If an autopsy is performed, there is little chance of overlooking cirrhosis as an underlying cause of death. Practically all patients dying from these

complications will consequently be officially reported as cases of liver cirrhosis.

There is a sharp rise in the number of newly diagnosed cases of liver cirrhosis in 1955–56 in C Region (Fig. 1 *a*). The peak is also seen in the selected material from the Medical Department of the University Hospital (Fig. 1 *b*). A rise in specific mortality began in 1956 and lasted for five years, with a peak in 1957 (Fig. 1 *c*). This increased specific mortality can also be observed in the other mortality curves (Fig. 1 *d* *e*). The changes are numerically small and in themselves of doubtful statistical significance. Analysis of the peaks shows, however that the increase is almost entirely due to an addition of middle-aged males from social group II. This very specific composition strongly suggests that the increase is real and attributable to a single factor The abolition of restrictions on the purchase of liquor in Sweden in 1955 may have had a wash-out" effect, influencing the epidemiology of liver cirrhosis in two ways. An increased alcohol consumption might aggravate subclinical cases of cirrhosis and give rise to an increase in newly diagnosed clinical cases. In severe cases it should result in an increase in specific mortality and perhaps in total mortality The especially exposed group would be middle-aged men among whom alcoholism is most common.

It thus seems reasonable to assume that the small but distinct rise observed in cirrhosis morbidity and mortality is real and due to the abolition of alcohol restrictions. The reasonableness of this assumption is strengthened by the fact that similar observations were made by Hällén et al. in Malmö (3 4) and by Engström in Stockholm (2) A similar phenomenon has been observed in alcoholics after discharge from long-time hospital care (20). During the first few years there is an increased mortality in liver cirrhosis among those patients.

A third effect to be expected from uncontrolled liquor consumption would be an increase in the total number of cirrhoses appearing after a certain delay As a matter of fact an increase in observed morbidity in C Region has occurred during the last 4–5 years. The increase is the same in men and women, and essentially concerns the higher age groups. The meaning of this observation is not clear It could, for example, be explained by changes in the composition of the clinical material or changes in the diagnostic facilities. It does not have the characteristic of an alcohol-induced increase as mentioned above. Further more a similar increase has also been observed in the Danish material.

In W Region there is an isolated peak in the morbidity curves for 1956 most evident in the series from the Central Hospital of Falun (Fig. 2 *a*, *b*). Probably this peak is of the same nature as the one found in C Region. During the whole period there is a continuous increase in the observed morbidity and mortality However the same trend can be seen in the observed incidence of malignant lymphoma. It thus seems most likely that the altered incidence of liver cirrhosis registered in W Region is essentially due to improved facilities for hospitalization and diagnosis.

An increased morbidity and mortality were registered in Århus in 1956–60 (Fig. 3 *a* *b*). The increase was quantitatively larger than the one observed in C Region. There was no sign of a sharp peak. The change was most pronounced among women. The reason for this change is completely obscure. There are no reports of any sudden (dramatic) changes in the drinking habits in Denmark. A possibility might be that the increased incidence of liver cirrhosis was a late sequel to the widespread epidemics of infectious hepatitis occurring during the 1940s. Two objections can, however immediately be raised against this possibility First, there is very little mention of previous hepatitis in the case histories. Secondly there are quantitative reasons the rate of cirrhosis developing after the hepatitis must have been within the order of several per cent, which is extremely unlikely (7 12).

Of course the observed increase may not necessarily be real. It could be explained by the increased autopsy rate together with an enhanced diagnostic activity

Assuming the increase to be at least partially real, it could be due to the observed change in the age distribution towards the higher age groups among the population of Århus. As liver cirrhosis in Scandinavia is primarily disease of old people, an increase in the number of elderly people in a hospital population will cause an increase in the observed incidence of liver cirrhosis.

A continuous moderate increase in total alcohol consumption has been observed in Denmark throughout the period of observation. A causal

Table I. *Incidence of cardiac rupture in autopsy population*

Authors	City or country	Year	No. of autopsies	Cardiac rupture No.	Cardiac rupture %
Meyer (13)	Munich	1854–1888	12 000–13 000	7	0.06
Meyer (13)	Leipzig	–1900	8 000	9	0.11
Turunen (23)	Helsinki	1880–1889	1 853	—	—
Krumbhaar and Crowell (10)	Philadelphia	1925	13 000	7	0.05
Edmondson and Hoxie (4)	Los Angeles	1924–1941	25 000	72	0.3
Wang et al. (24)	Boston	1926–1945	7 018	23	0.33
Friedman and White (6)	Boston	1935–1940	2 947	10	0.34
Wessler et al. (25)	Boston	1936–1950	1 641	19	1.2
Oblath et al. (18)	Los Angeles	1941–1951	13 665	80	0.58
Zeman and Rodstein (27)	New York	1942–1955	668	16	2.4
London and London (11)	Miami Beach	1951–1964	3 416	47	1.4
Möttönen (16)	Finland	1964–1965	5 025	40	0.8

same in the last 40 cases. After the age of 40 the prevalence seemed to increase rapidly and in the age group 40–45 it was 50%.

Although the criteria especially of mild coronary sclerosis may vary, it seems, on the basis of the figures above, that the prevalence of coronary sclerosis in young Finnish men is nowadays distinctly lower than in the US soldiers in the Korean War and most likely also lower than in German soldiers in World War I. This seems to be true despite the fact that the *official* coronary mortality in Finland is to-day internationally very high.

It is concluded that evidence for increase in the prevalence of coronary sclerosis in the present century is lacking.

ANGINA PECTORIS

Although the clinical picture of angina pectoris has been known in its present form for more than 200 years, knowledge of the prevalence of this syndrome has been lacking until the population studies of recent decades. Thus, when discussing the prevalence of the syndrome, we have to use indirect evidence, first of all the interest shown in it in the literature.

The oldest description of angina pectoris is probably that in the Papyrus Ebers (3). Hippocrates also knew the syndrome though the reverse is often claimed (1). The description by Erasistratus of chest pain that makes a walking man halt, after which he can walk on again, is characteristic (21). The blind philosopher Seneca's disease "suspirium" was no doubt angina pectoris, too.

When Heberden published his classical description of angina pectoris, interest in this syndrome had already been awakened. Before Heberden, it had been described, e.g., by Bartoletti (1633), Poterus (1645), Ballonius (1735), Lancisi (1738), Sauvages (1763), and Morgagni (1765) (e.g. 8, 21). In the same year as Heberden's investigation was published (1768), Rougnon described the disease as well, and this led to a national dispute of priority for decades (8).

After Heberden, the interest in angina pectoris was obviously lively. Forbes (5) wrote in 1838: "After Heberden, numerous physicians in England, France, Germany and Italy described this disease (angina pectoris) both in journals and in separate publications. After the attention of the medical profession was drawn to this syndrome, it appears to have become their pet and merely to list cases reported and monographs written would fill many pages." (5). In 1927 Kohn (8) listed 57 papers on angina pectoris from the years 1772–1807.

Forbes' list of synonyms (5) also gives a good picture of the wide interest.

Cardiogmus cordis sinistri	Sauvages 1763
Angina pectoris	Heberden 1768
Die Brustbräune	Elsner 1780
Diaphragmatic gout	Butter 1791
Asthma arthriticum	Schmidt 1795
Syncope angens	Parry 1797
Asthma dolorificum	Darwin 1801
Stenodynia syncopalis	Sluis 1802

Asthma spastico-arthriticum inconstans	Stoeller 1803
Suspirium cardiacum	Stephen 1804
Sternalgia	Baumer 1806
Stenocardia	Brera 1810
Pnigophobia	Swediaur 1812
Angor pectoris	Frank 1818

As we remember how small was the number of doctors, medical periodicals, and investigations published in them, at that time, we see that the interest in angina pectoris during the decades after Heberden must have been really lively. It is not likely that a rare disease would have had such an attraction. The size of the materials also shows the prevalence of angina pectoris. Heberden's own material, for example, consisted of 100 cases, and Forbes' clinical material of 88 and autopsy material of 45 cases.

Osler (19) was obviously the first to discuss the possible increase of angina pectoris. He asked in his Lumleian lectures in 1910: Has angina pectoris increased in the community? Has the high pressure of life of modern days made the disease more common? There is an impression among consultants in the United States that there has been an increase of late years, a view not borne out for this country by the figures available. The question thus remains unanswered by Osler. Moreover, he defined angina pectoris rather widely including at least partly myocardial infarction in the diagnosis "status anginosus".

Later on, the pertinent literature does not discuss possible changes in the prevalence of angina pectoris. Based on Heberden's material, Kristensen (9) alone has recently tried to compare the prevalence of angina pectoris in Heberden's London with that of the present-day Norway. He comes to the conclusion that the prevalence has probably been the same.

MYOCARDIAL INFARCTION

Hospital and autopsy materials show that the incidence of myocardial infarction has increased during recent decades. Is this increase true? An observation time of sufficient length should be needed in order to solve the problem. Information on the incidence of myocardial infarction in the 19th and at the beginning of this century is lacking because the disease was earlier unknown to physicians and pathologists.

Cardiac rupture is a complication of myocardial infarction that has always been observed in autopsies. On the basis of the incidence of cardiac rupture, some conclusions of the incidence of myocardial infarction can be drawn.

Table I presents the incidence of cardiac ruptures in hospital autopsy materials during 100 years.

As can be seen, the incidence of cardiac ruptures *during 100 years has increased ten- to twentyfold*. When analysing the increase it must be kept in mind that the materials are unselected hospital materials, where the incidence of myocardial infarction and thus also of cardiac ruptures depends on several factors. Although the possible increase of the incidence of myocardial infarction could be excluded, there are still several other factors that cause an erroneous increase.

1. As long as myocardial infarction was unknown, patients suffering from this disease did not go to physicians or hospitals. The physicians neither sent their patients to hospitals, nor were they admitted. It is difficult to imagine that at the beginning of this century an attack of chest pain lasting for a couple of hours would have been a reason to call a doctor or be admitted to a hospital. Doctors were few and hospital beds still fewer and chiefly reserved for infectious diseases.

It is interesting to note in Table I that, from the 1930's onwards, the relative increase of cardiac ruptures is only about threefold, though myocardial infarction was then still unknown to laymen, and only poorly known by physicians.

2. A great change has taken place in the age distribution of the population and hospital patient materials. The same is even more true of the age distribution of hospital autopsy materials. Since autopsies were not performed on all, the tendency was to do so primarily in young and "interesting" cases. Thus, in the autopsy material of the Pathological Institute of Helsinki University 75 % were under 50 years of age in 1885 and only 10 % in 1965 (23). In the Radcliffe Infirmary material in Oxford 51 % were under 45 years in 1930 against 17 % in 1960 (20).

The incidence of cardiac ruptures associated with myocardial infarction increases with the age of the patient. To-day a rupture is rare in patients under 50 years of age. None of Friedman and White's (6) and Möttönen's (16) 130 pa-

tients was younger than 50 years. Two ruptures of *Edmondson and Hoxie's 72 cases (4) occurred* in patients under 50 years, whereas 9 6% of the 865 myocardial infarction patients in the same material were younger than 50 years. Earlier cardiac ruptures seem to have been more common in young patients. Seven (16%) out of 44 were under 50 years of age in Meyer's material (13). Two out of these seven had a typical infarction history the personal history being lacking in four cases. Krumbhaar and Crowell's large material of 654 cardiac ruptures during 1872–1922 included 11% of patients 30 to 50 years old (10).

The incidence of cardiac ruptures in sudden deaths seems to have remained unchanged since the end of the last century According to Devergne (2) there is one cardiac rupture among every 40 sudden deaths. About 60 years later Martland (12) found 42 ruptures in 2 000 sudden deaths. The incidence of cardiac ruptures was 43 (3.6%) in the 1 213 sudden deaths of Wiklund in Stockholm in 1955–1966 (26). This material consisted, however of many old patients, 40% being older than 70 years. In sudden deaths of patients under 70 years in this material the incidence of cardiac ruptures was 2.3% no ruptures were noted in patients under 50 years.

Besides cardiac ruptures, the schwielig myocarditis, especially well known by German pathologists at the end of the last century may be considered to reflect to a certain extent the incidence of old infarct scars in autopsy materials. This finding was obviously not rare for example, Eulenburg's famous Real Encyclopädie der Gesamte Heilkunde mentions in 1887 that this kind of *change in cardiac dilatation and hypertrophy can* be observed even with the naked eye so häufig More accurate statistical data are unfortunately lacking.

COMMENTS

It can be concluded that evidence for an increase of the prevalence of coronary sclerosis or angina pectoris is lacking.

Increase of the cardiac rupture is distinct in hospital and autopsy series but lacking in patients with sudden deaths outside hospital. As stated above, it is probable that the increase of cardiac ruptures is not real. At any rate figures of cardiac ruptures show that the incidence of myocardial infarction cannot have been very low at the end of the last and beginning of the present century

We readily accept the statement that there are more coronary risk factors in the society of to-day than earlier This is certainly true, at least partly for example greatly increased cigarette smoking is one of them. On the other hand, we often forget that life is easier to-day for a great part of the population and perhaps less stressful than before, which possibly decreases the risk of coronary heart diseases.

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HUMAN EXPOSURE TO MERCURY FROM GOOSANDER EGGS CONTAINING METHYL MERCURY

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Abstract A woman living in the Aland Islands in the Baltic had symptoms and signs which caused suspicion of mercury poisoning. She was found to be exposed to mercury and the mercury was traced to goosander (Mergus merganser L.) eggs which she had consumed every year. Goosander eggs, 36 in all, were collected from various parts of the islands and were found to contain between 0.3 and 3.5 mg/kg of mercury (mean 1.4 mg/kg). Between 0.5 and 5.8 mg/kg was found in the whites (mean 1.9) and between 0.2 and 1.8 mg/kg in the yolks (mean 0.7 mg/kg). The main part of the mercury (mean 78% of the total mercury) was in the form of methyl mercury. The mean weight of the eggs without shell was 71.1 g. The fish in the waters of the Aland Islands have been found to contain only negligible amounts of mercury whereas the fish in the birds' winter quarters (the Danish sounds and southern Baltic) have been found to be contaminated with this metal. It is concluded that the birds are contaminated in their winter quarters and carry the mercury to their summer quarters, where contaminated eggs are consumed by the inhabitants. To the best of the authors' knowledge, this is the first report of migratory birds carrying pollutants from their winter quarters and causing risk of human exposure to the pollutant in the summer quarters.

Human exposure to mercury in hen's eggs is well known. Such cases were previously not infrequently observed, especially as long as alkyl mercury was used for seed dressing. Lately the risk of this form of exposure has become less obvious because these dressing compounds have been prohibited by many authorities. The risk of exposure to mercury is now mainly due to contamination of water from industrial outlets, with incorporation of mercury into nutrition chains. It is also known that inorganic mercury may be alkylated in nature, and this augments the risk of exposure to mercury in alkylated form.

Recently we observed a patient who had symptoms and signs which suggested the possibility of mercury poisoning. She was found to have been exposed to mercury and it subsequently became obvious that she had eaten mercury-containing goosander (Mergus merganser L.) eggs.

The patient lived in the Aland Islands, which are situated around latitude 60° N in the Baltic between the mainlands of Sweden and Finland. They consist of the main island of Aland, about 6 000 larger islands and innumerable smaller islands, rocks and skerries. The number of resident inhabitants is about 22 000.

During the egg-laying season, goosander eggs are commonly eaten by inhabitants of the area who have access to land in the outer skerries. The goosander accepts nest boxes which are put out facing the sea, and the eggs are collected from the boxes and considered to be a delicacy. The goosander is a "layer" in a way resembling that of poultry and thus collection of several eggs from the same nest box is possible.

CASE REPORT

A 45-year-old housewife from Eckerö was admitted to hospital on April 28 1970 because of paraesthesia in her lower limbs. The paraesthesia had persisted for about one year beginning in the spring of 1969. It had then consisted of a sensation of needle-pricking over her whole body and of numbness of the feet. At the same time there had been visual disturbances in the form of difficulties in reading due to "jumping" of the lines. Eye-glasses had been tried but did not help. During 1969 and in the beginning of 1970 the paraesthetic symptoms had become worse and concentrated to the lower limbs. She saw a doctor who reassured her and stated that the symptoms are of neurotic origin.

There were no special circumstances in the patient'

Fig. 1 Map of the Åland Islands. The dots correspond roughly with the exact site where each goosander egg was collected.

surroundings, except for the fact that as long as she could remember she had eaten about 20 or more goosander eggs every spring. She did not eat any appreciable amount of meat of fish-eating game-birds. No painting had been done in the home for several years, *no chemicals were used, and there had been no mer*curial compounds in the house. No drugs except occasional sedatives had been used. Mercurial seed dressing had not been used in this region.

The patient's general condition was fair. She had a small nodular goitre. General physical examination revealed nothing special except E. coli pyelitis. There were no gum changes. Neurological examination revealed fine tremor of the fingers. There was diminished pain and touch sensibility of the whole left fore-arm. Touch sensibility was diminished over the lateral aspect of the left foot, *touch and pain sensibility was diminished over the* medial aspect of the left calf. The static, ocular fundi and perimetry were normal.

The haematological values and PBI were normal. At admission the patient excreted an average of 40 μg of mercury per day in the urine (analysis at the Institute for Occupational Health, Helsinki; normal value below 20 μg/day). Her hair contained [illegible] mg of mercury per kg, which is moderately elevated value (activation analysis at the Reactor Laboratory of the Technical University Helsinki; normal mean value 1.5 mg/kg). The blood was not examined for mercury at this time. Chromosome analysis (Medix Clinical Laboratory Helsinki) showed no signs of chromosome damage and no chromatide breaks.

It was concluded that the patient had been exposed to mercury at some instance, although the symptoms and signs did not definitely corroborate a diagnosis of manifest chronic mercury poisoning. She was given dimercaprol intravenously and excreted altogether 500 μg of mercury during series of ten daily injections of 100 mg each. She was then discharged from hospital and further supervised in her home.

As no possible source of mercury could be found in the patient's environment except for the yearly intake of goosander eggs, it was decided to study whether goosander eggs contained any amounts of mercury and especially methyl mercury

METHODS

With the aid of coast guard personnel and of the district nurses, 37 goosander eggs were obtained from different *nest boxes in various parts of the skerries of the Åland* Islands, as shown in Fig. 1.

For determination of total mercury the eggs were analysed by an oxygen-flask combustion-diffusion method (4). This method has been cross-checked with neutron activation analysis for determination of total mercury in biological samples, and the results have corresponded satisfactorily (7). At low levels, however many elements are known to interfere with the results. It must also be noted that, in analyses of eggs, the yolk which is rich in lipids may undergo incomplete combustion. The carbonization may then cause inexactness of the results. The sensitivity of this method is 0.1 mg/kg wet sample

For determination of CH_3-bound mercury gas chromatography in combination with an electron capture detector was used (5). In this determination the methods of cleaning are important. In analyses of eggs no source of error was found. For control purposes water solution of methyl mercury dicyandiamide and benzene-toluene solution of methyl mercury chloride were subjected to

the same cleaning and analysing treatment as the eggs. The greatest deviations at the level 0.5 mg/l were ±5%.

All the eggs were fresh when analysed, except for six hich were moderately decomposed. The white and the yolk of each of the fresh eggs were analysed separately hereas the decomposed eggs were analysed in toto after shelling.

RESULTS

The results of the analyses are shown in Tables I and II. All the eggs contained mercury the main part of which was methyl mercury Of the total mercury content a mean of 89% in the whites, 69% in the yolks and 78% in the whole eggs

Table I. *Basic facts about the eggs*

No.	Parish	Weight (g) Total	White	Yolk	White + yolk
1	Eckerö	80.8	33.8	36.1	69 9
2	Eckerö	82.0	40.5	30 6	71 1
3	Eckerö	85.8			73.7
4	Eckerö	82.6	30 6	40 0	70 6
5	Föglö	85.5	34 0	36.9	70.9
6	Föglö	83.0	34.3	37.5	71 8
7	Föglö	79.0	30.9	38.2	69 1
8	Föglö	91.5	39 7	39.2	78.9
9	Föglö	91.0	41.0	35.2	76.2
10	Sottunga	76.7	36.8	30 6	67 4
11	Sottunga	82.1	37.3	34.5	71.8
12	Sottunga	81.8	34.1	37.3	71 4
13	Sottunga	83.2	35.8	35.3	71 1
14	Sottunga	88.2	39.9	37 4	77.3
15	Sottunga	83.3	34.8	35.2	70.0
16	Sottunga	88 4	39.0	36.7	75.7
17	Brändö	81 5	30.5	39.9	70.4
18	Brändö	87.5	34.6	35.4	70.0
19	Brändö	81.8	36.7	34 9	71.6
20	Brändö	84.9			73.5
21	Brändö	73.3	26.0[b]	38.7[b]	64 7
22	Brändö	77.9	30.4	38 9[b]	69.3
23	Brändö	81 4	35.1	36.3	71 4
24	Brändo	79.3	29 4	36.4	65.8
25	Brändö	72.7	32.1	26 5	58.6
26	Brändö	93.9	39.2	42.5	81.7
27	Brändö	89.5	38 6	39.2	77.8
28	Brändö	85.6	38.8	36 0	74 8
29	Kökar	77.8			67.5
30	Kökar	77.3			66.8
31	Kökar	72.9			61.5
32	Kökar				14 5
33	Kökar	85.2	35.3	39.9	75.2
34	Kökar	76 6	31.1	35.4	66.5
35	Kökar	85.8	41 3	32.3	73 6
36	Kökar	84 8	43.3	30.2	73.5
37	Kökar	85 4	38.6	31.5	70.1

White and yolk could not be separated.
[b] Admixture of white to the yolk.
Crushed—only parts recovered for analyses.

Table II. *Results of mercury determinations*

No	Total mercury (mg/kg) White	Yolk	Total	CH_3-bound mercury (mg/kg) White	Yolk	Total
1	2.0	1.8	1.9	2.67	0.88	1.74
2	1.3	0.7	1.1	2.38	0.49	1.56
3			0 9	Not analysed		
4	3.8	0 9	[illegible]	4.49	1.31	2.69
5	2.5	1.0	1.7	1.39	0.03	0.68
6	2.2	1.8	2.0	1.29	0.52	0.89
7	0.5	0.9	0.8	0.28	0.43	0.36
8	1.0		1.0	0.56	0.21	0.39
9	1.7	1 1	1.0	2.18	0.33	1.33
10	5.8	1 1	3.7	3.83	1.28	2.67
11	2.3	0.5	1.4	[illegible]	0 64	1.57
12	2.4	0 3	1.3	1.65	[illegible]	0.85
13	1.9	0.7	1.3	2.02	0.58	1.31
14	0.8	0.2	0.5	0.51	1.03	0.76
15	0.7	0.3	0.5	0.50	0.16	0.33
16	0.6	0.2	0.4	0.32	0.17	0.25
17	1.8	0.9	1.3	[illegible]	0.78	1 49
18	1.0	0.1	0.5	0.57	0.20	0 38
19	3.6	0.7	[illegible]	3.00	0.81	1.93
20			[illegible]	Not analysed		
21	0 8	0.5	0.6	0 70[b]	0.41	0.53
22	0.3	0 3	0.3	0.52[b]	0.28	0.39
[illegible]	1.5	0.7	1 1	2.13	0.68	1.39
[illegible]	1 7	0.9	1.3	1.98	0.32	1.06
25	3 4	0.4	2.0	3 19	0.84	[illegible]
26	2.8	1.8	1.9	1 17	0.31	0 72
27	3.7	1.0	2.4	4 47	0.33	[illegible]
28	0.8	0.3	0 6	0.33	0.16	0.25
29			3.5	Not analysed		
30			[illegible]	Not analysed		
31			2.1	Not analysed		
32			1.2	Not analysed		
33	2.2	0.3	1.2	1.31	0.36	0.81
34	1.9	0 9	1.4	1.66	0.79	1.20
35	1 4	1 1	1 3	1.26	0 48	0.92
36	1.3	0.3	0.9	0.78	0.13	0.51
37	1.5		1.5	0.88	0.14	0.55
Mean	1.9	0.7	1.4	1 71	0.49	1.12
	31 eggs		37 eggs		31 eggs	

[a]. See Table I.

was in the form of methyl mercury The range of total mercury was 0.3–5.8 mg/kg in the whites, 0.3–1.8 mg/kg in the yolks and 0.3–3.5 mg/kg in the whole eggs. The range of CH_3-bound mercury was 0.28–4 49 mg/kg in the whites, 0.03–1.31 mg/kg in the yolks and 0.25–2.69 mg/kg in the whole eggs.

DISCUSSION

The patient

It was shown that the patient had been exposed to mercury although it cannot be stated that

there was genuine mercury poisoning It is obvious that the mercury in the patient, at least mainly originated from the goosander eggs, of which she had consumed a score or more every year for several years. This is further corroborated by the fact that no mercury could be detected in the urine from people who lived in the same village as the patient, but who had not eaten goosander eggs.

If the mercury content of the eggs consumed by the patient had been of the same magnitude as in the eggs analysed by us, then it may be calculated that she had ingested roughly between 0.5 and 6 mg of mercury (between 0.4 and 4.5 mg of methyl mercury) every season as long as the mercury content of the eggs had been of the present magnitude. This would mean that her body retained roughly between 100 μg and 1 mg of CH_3-bound mercury (1).

It is always difficult to assess whether symptoms such as the patient's are a consequence of poisoning or derive from other causes in a person who is exposed to mercury The patient's complaints and the clinical signs, as well as the mercury values from urine and hair do not rule out mercury poisoning. We do not wish to take a definite stand for the presence of mercury poisoning in our patient, but exposure to mercury is obvious. The presence or absence of poisoning is a consequence of dosage, and this question is of minor importance in this paper

The goosander eggs

The eggs contained amounts of mercury and of methyl mercury well above the upper limit of 0.05 mg/kg suggested by the WHO for mercury in food, and also above the limit of 0.5 mg/kg stated by the Canadian and US authorities, and of 1 mg/kg by the Swedish authorities, for mercury in fish used as human food. It is obvious that eggs which contain such amounts of mercury as those which we analysed should not be used as human food.

The fact that some eggs are shown to contain more methyl mercury than total mercury is explained by the fact that the analysis for total mercury not infrequently results in too low values (see Methods) Such discrepancies are often present in analyses of this type, also in neutron activation analysis.

The eggs were collected from many different islands and from different nest boxes on each island. The amounts of mercury can thus not be regarded as a consequence of strictly local factors.

The goosander is a pure fish-eater and it has been shown previously (6) that this bird's pectoral musculature contains more mercury than those game-birds' which feed on vegetable or mixed food. The goosander is migratory The birds which nest among the Åland Islands have their winter quarters in southern Sweden, the Danish sounds, the southern Baltic and in rivers, lakes and coastal waters of Central and Western Europe (2), i.e. parts of the world where pollution from industrial mercury is common. It has been shown (3) that fish from Danish waters is heavily contaminated with mercury On the other hand it has been shown (7) that fish from the waters around the Åland Islands contain only very small amounts of mercury There are no big industries on the Åland Islands, nor any small industries which work with mercurial compounds. Mercurial seed dressings are not used. It is thus not unreasonable to postulate that the goosander is contaminated with methyl mercury from fish in its winter quarters. The mercury is then retained in the birds bodies, and subsequently this results in contamination of the eggs in the birds' summer quarters, where eggs are collected and eaten and thus may constitute a potential health hazard. The shortness of the egg-laying period, i.e. only a few weeks every spring, diminishes the health risks for the consumers. Goosander eggs may of course, without difficulty be removed from the list of human foods.

The occurrence of substantial amounts of mercury in goosander eggs, with risk of exposure to mercury for the consumers of these eggs, is as far as we know the first instance of migratory birds being demonstrated to carry a risk of pollution to the human environment in their summer quarters through poisonous substances from their winter quarters. We have discussed this in a previous short communication (8). It is obvious that inadequate control of environmental pollution anywhere in the world under unfavourable circumstances may cause similar problems elsewhere, if the pollutants are carried by migratory birds from their origin to other parts of the world.

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Congress Announcements

The Third International Congress of Psychosomatic Medicine in Obstetrics and Gynaecology will be held in London, March 29 to April 2, 1971

Organisation Committee
Sir John Peel, President, Professor Norman Morris, Chairman, Dr Leon Chertok, Secretary General.

Secretariat
Kurt Fleischmann & Associates, Chesham House 136 Regent Street, London W1 England.

The Second Congress of the European Association of Radiology will be held in Amsterdam, the Netherlands, June 14 to 18 1971

Secretariat
Before and after the congress: c/o Holland Organizing Centre, 16 Lange Voorhout, The Hague, the Netherlands.

During the congress: International Congress Centre RAI, Europaplein, Amsterdam, the Netherlands.

The Seventh International Congress of Chemotherapy will be held in Prague, Czechoslovakia, August 23–28 1971 The official congress language is English, without simultaneous translation.

Secretariat
Up to August 21 1971 VIIth International Congress of Chemotherapy Soloská 31 Prague 2. After August 21 1971 House of Arts (Rudolfinum), Nám. Krasnoarmějců, Prague 1 Czechoslovakia.

The Sixth International Congress of Physical Medicine will take place at Palacio de las Naciones, Barcelona, Spain, 2–6 July 1972.

Secretariat
Calle Ravella, 4 Barcelona 6 Spain.

Les Journées Médicales Annuelles de L'Hôpital Broussais – La Charité, service du professeur Paul Milliez, auront lieu 4–6 Mai, 1971 Journées consacrées aux acquisitions médicales récentes.

Il est recommandé de s'inscrire assez tôt, le nombre des participants étant limité. Prière d'envoyer les droits d'inscription au Centre de recherches sur l'hypertension artérielle, Professeur Milliez, Hôpital Broussais, 96 rue Didot, Paris-XIV• France.

Actualités Néphrologiques de L'Hôpital Necker 7–9 Mai, 1971

Secrétariat
Département de Néphrologie, Hôpital Necker 161 rue de Sèvres, Paris-XV France.

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INFLUENCE OF RECIRCULATION ON MYOCARDIAL CLEARANCE CURVES WITH XENON 133

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Abstract. Myocardial clearance curves with inert radioactive gas as indicator regularly deviate from monoexponential curves. When Xenon is used as an indicator and *injected into the left heart the major part* is removed from the circulation by effective alveolar ventilation, while a minor fraction re-enters the left heart and recirculates through all vascular beds including the coronary vascular system. The effect of this recirculation on the shape of the myocardial clearance curve has been investigated under conditions when deviation is especially pronounced, i.e. pacemaker-induced tachycardia. Tachycardia causes increased coronary flow with steeper clearance curve, while the ventilation and hence the amount of recirculating Xenon is unchanged. The resulting marked deviation is significantly diminished by voluntary hyperventilation, which decreases the recirculation. This explains the decrease of deviation during physical exercise when both coronary flow and effective alveolar ventilation increase. The error imposed by recirculation on the calculation of coronary flow is on average less than 5% during pacemaker-induced tachycardia.

Clearance curves *describing regional blood flow* are easy to analyze when they follow an ideal monoexponential function. Most tissues, however give clearance curves that are more complex. This has been explained by inhomogeneous flow within the volume of distribution of the indicator and by tissue components with different partition coefficients for the indicator used (3 7 13). In most published works presenting myocardial clearance curves it is observed that they also deviate from the expected monoexponential function curve. Despite this the curves have often been analyzed as if they represented a homogeneous mass and flow (5 8, 10, 11). During the study of coronary flow with the indicator Xenon 133 the same deviation from the ideal curve has previously been observed (6). Recirculation was considered a possible major factor in this phenomenon. During subsequent coronary flow studies on patients with pacemaker-controlled heart rates, even greater deviation from the ideal monoexponential curves was observed. The present study was designed to determine whether recirculation could also explain this increased deviation and whether these complex clearance curves could be used for estimation of coronary flow

MATERIAL

Twelve patients are included in group I, seven with ischemic heart disease and five without cardiac disease. The heart rate was varied with graded exercise. All patients in this group had normal sinus rhythm. Twenty patients are included in group II. In this group heart rate was changed with a cardiac pacemaker. Thirteen patients had third degree AV-block, one second degree AV block, and six a normal conduction system.

METHODS

All patients were investigated in the fasting state, without premedication and in the supine position. A no. 6 Eppendorf catheter was positioned in the coronary sinus with the tip approximately 4 cm from the ostium. The position of the catheter was confirmed by the injection of a small amount of contrast medium. A second catheter was placed in the left atrium by the transseptal technique. A polyethylene-catheter (PE-205) was inserted in the right brachial artery

In the group II patients a bipolar electrode catheter (USCI no. 5651) was also inserted in the right basilic vein. In those with AV-block the tip was placed in the right ventricle, and in those with a normal conduction system in the right atrium. An Elema model 138 pacemaker with variable frequency and amplitude, was employed for pacing. A scintillation detector with a 2-inch NaI crystal and a narrow cone collimator was placed over the lateral right lung. The collimator was positioned

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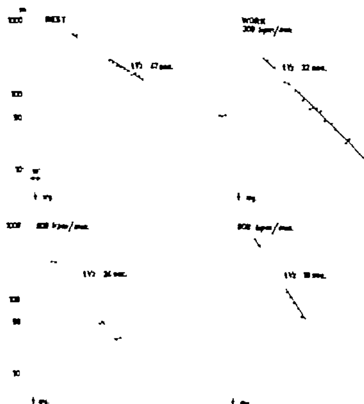


Fig. 1. Myocardial clearance curves from one patient at rest and during increasing levels of physical work. In all clearance curves a slight terminal deviation of the curve can be seen.

to avoid contamination by heart radioactivity. Exercise was performed on a bicycle ergometer (Elfa-Elema) with the patient remaining supine.

Coronary blood flow (CF) was measured by the clearance method using Xenon 133 as an indicator. 0.5 mC was dissolved in 2 ml NaCl and injected rapidly in the left atrium through the transseptal catheter. The gamma-radiation from Xenon was continuously measured in coronary sinus blood. This Xenon technique for measuring coronary blood flow has been described in detail elsewhere (6).

Gamma-radiation in the coronary sinus blood was plotted at 10 sec intervals on a semilog scale and the down-slope fitted by eye. Half-times were calculated from the initial slope, and no attempt was made to analyze the curves as representing a multi-compartment system. All clearance curves terminally deviated from an ideal mono-exponential curve, and the point of deviation was readily defined. The activity level at that point is presented as per cent of maximal activity for that curve. Figs. 1 and 2 are typical examples.

To evaluate the recirculation of Xenon, the isotope was injected in the descending aorta at the diaphragmatic level. Thus no indicator reached the coronary capillary bed primarily but circulated first via the peripheral and the pulmonary capillary bed. *Any Xenon activity appearing* in coronary sinus blood must then represent recirculation to the left atrium as modified by passage through the myocardium.

Determination of the recirculation by the above technique was performed on many of the group II patients. Each time it was preceded by a coronary blood-flow measurement. Both these procedures were as far as possible carried out under identical conditions. This schedule was followed for various combinations of rest, work, hyperventilation and pacing.

A 10 min interval separated each study. Stable and similar conditions were sought for each schedule. In all

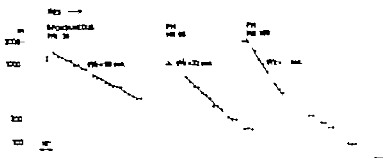


Fig. 2. Myocardial clearance curves from a patient with AV-block III. All curves recorded at rest, one during spontaneous heart rate and two during different pacemaker-induced higher heart rates. In the two latter a marked late deviation of the curve can be seen.

recirculation studies the heart rate was kept constant with pacemaker overdrive to assure comparable conditions during the coronary blood flow and the subsequent recirculation measurement. In order to get an approximate measure of the degree of ventilation during hyperventilation studies, the isotope activity was recorded over the lateral part of the right lung.

Assuming identical hemodynamic and ventilatory conditions for the successive procedures, recirculation activity was subtracted from the clearance curve to get "corrected" clearance curve.

RESULTS

Deviation of Wash-out Curves

Patients in group I were investigated at rest and at work. The clearance curve half-time, $t^1/_2$, in this group varied from 14 to 49 sec. Each clearance curve deviated from the initially linear slope at a level between 3 and 20% of the maximal activity (Table I). In most patients with more rapid clearance curves, the deviation occurred at a lower level. This is shown in Fig. 1 and is most apparent with the shift from rest to work.

Table I. *Deviation of clearance curves in group I*

Pat.	Work load (kpm)	Initial $t\frac{1}{2}$	Deviation of clearance curve at % of max. activity
1	100	27	15
	200	23	13
	300	21	17
2	150	20	17
	300	15	8
	450	14	7
3	200	27	19
	450	20	3
	600	18	7
4	150	31	7
	300	28	6
	450	26	7
5	150	26	7
	300	22	4
	450	18	7
6	150	27	8
	350	19	8
	500	15	3
7	200	27	13
	400	27	6
	600	21	5
8	rest	49	15
	200	23	8
	400	22	4
	800	17	4
9	rest	47	12
	300	32	5
	600	24	7
	900	18	8
10	200	32	11
	400	24	6
	600	16	7
11	100	22	9
	200	18	6
	300	16	—
12	200	20	9
	400	15	10
	500	14	— <

Group II patients were investigated at rest, first at spontaneous heart rate and then with pacemaker-controlled rates between 60 and 150 beats per min. Clearance curves showed a half-time between 18 and 54 sec (Table II). The clearance curves deviated from a linear slope at between 8 and 35% of the maximal activity

Table II. *Deviation of clearance curves in group II*

Pat.	Heart rate	Initial $t\frac{1}{2}$	Deviation of clearance curve at % of max. activity
13	41[a]	54	7
	72[b]	48	0
	98	34	21
14	56[a]	33	24
	85[b]	23	25
15	72[a]	35	21
	85[b]	32	13
	98[b]	30	7 <14
16	60[a]	54	35
	100[b]	34	16
	150[b]	24	20
17	38[a]	30	24
	85[b]	32	21
	150[b]	18	23
18	26[a]	43	0
	85	24	19
	98	21	16
19	45[a]	51	0
	85[b]	30	11
	158[b]	22	7
20	100[a]	33	24
	150[b]	33	26
21	45[a]	45	7
	72[b]	35	25
	100[b]	28	22
22	70[a]	44	9
23	70[a]	39	15
	100[b]	30	17
	150[b]	24	21
24	90[a]	43	18
	100[b]	42	11
	150[b]	32	16

[a] Spontaneous heart rate.
[b] Pacemaker overdrive.

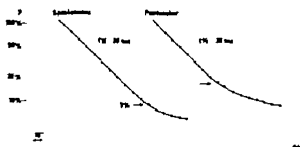


Fig. 3 A schematic illustration of the experimental findings. For a given coronary flow the deviation starts higher on the clearance curve in the group in which the heart rate and accordingly the coronary flow is increased with pacemaker.

Thus groups I and II differ in respect of the point where the initially linear slope is broken. This point comes at a higher activity level in group II (Fig. 2).

Because the point of curve deviation is related to coronary flow (or $t^1/_2$), it is most appropriate to compare groups with similar flows. Thus only clearance curves with half-times of 20–40 sec are included. With this limitation the extremes of coronary flow are avoided. In the more limited groups the mean value for the initial curve deviation is 9 and 19% for groups I and II, respectively. This means that, for any given coronary flow the deviation starts higher when the heart rate, and accordingly the coronary flow is increased with pacemaker. This is illustrated schematically in Fig. 3

Recirculation

When the Xenon is injected in the descending aorta the radioactivity rises slowly in the coronary venous blood to a maximum approximately 2 min after injection. This is 60–90 sec later than the maximum of the corresponding clearance curve. It reaches 2–5% of the maximum activity of the clearance curve and then slowly falls. The half time of the recirculation activity curve is 180 to 600 sec, representing a very slow activity decrease.

Influence of Recirculation Activity on Clearance Curve

A. At rest

Eight patients in group II were studied at rest. In all the heart rate was kept constant with pacemaker overdrive at 100 beats/min. Two patients had normal conduction system (nos. 25 and 32). The other six had third degree AV-block. An example of such a measurement is shown in Fig. 4. In this case the recirculation activity was 2.4% of the maximal activity in the preceding clearance curve. The corrected clearance curve is also shown. Similar recirculation values are observed in the other cases. Table III presents the maximal activity of recirculation expressed in per cent of the maximal activity of the preceding clearance curve. The recirculation curve recording from coronary sinus blood has a uniformly horizontal configuration in all cases. The $t^1/_2$ varied between 181 and 600 sec. In some cases it has not been possible to calculate the $t^1/_2$ because of the low level of activity.

B. Work

The recirculation was investigated in three patients, both at rest and during work. The heart rate was kept constant by pacemaker. In all three

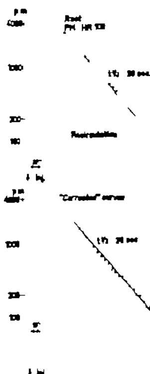


Fig. 4. A clearance curve and a subsequent recirculation curve with the patient at rest and the heart rate increased with pacemaker. The "corrected" curve is constructed by subtraction of the recirculation curve from the clearance curve.

Table III. *Recirculation at rest*

Pat.	Max. activity clearance curve (cpm)	Max. activity recirculation curv (cpm)	Max. activity recirculation / Max. activity clearance curve (%)	$t_{1/2}$ recirculation
25	1 000	50	5.0	470
26	1 800	35	1.9	181
27	1 700	40	2.6	222
28	1 900	40	1.8	—
29	3 000	100	2.6	263
30	2 500	110	4.4	600
31	2 400	110	4.6	243
32	1 500	65	4.3	—
Mean			3.5	
S.D			1.3	

patients the recirculation activity was lower at work than at rest in the later part of the curve where the influence on the clearance curve is biggest (Table IV). Fig. 5 shows the results from one patient, and also the corrected curve.

C. *Hyperventilation*

The recirculation was measured in three patients at rest, both with normal respiration and during hyperventilation. The heart rate was kept constant with pacemaker. Fig. 6 shows typical results from one patient. In all the patients hyperventilation lowers the activity level of recirculation to less than half of that recorded during normal respiration (Table V). Fig. 6 also demonstrates that there is a greater difference between the original and the corrected clearance curve with normal respiration than with hyperventilation.

The isotope activity recording over the lateral portion of the right lung gave similar activity levels in comparable studies, indicating that the degree of ventilation and hyperventilation, respectively was the same during the coronary flow and the subsequent recirculation measurement. Fig. 7 shows the results for one patient, and Table VI gives lung activity levels for the two patients in whom they were measured.

Quantitative Estimation of the Influence of Recirculation on the Myocardial Clearance Curve

The demonstrated recirculation activity is of a magnitude which affects myocardial clearance curves. This effect is of most significance late in the clearance curve when the relatively constant level of recirculating Xenon becomes a larger proportion of total activity. However even the initial portion of the clearance curve used for coronary flow calculation is influenced, resulting in spuriously elevated values for coronary flow. The magnitude of this change can be calculated from the recirculation studies.

Comparison of the $t_{1/2}$ value of the corrected curve with the uncorrected value quantitates the

Table IV. *Recirculation at rest and during work*

Pat.	Work load (kpm)	Deviation of clearance curve at % of max. activity	Max. activity recirculation / Max. activity clearance curve (%)	$t_{1/2}$ recirculation
27	Rest	20	2.6	222
	200	29	2.9	155
28	Rest	27	1.8	∞
	0	16	0.9	131
29	Rest	18	2.6	263
	100	6	1.6	231

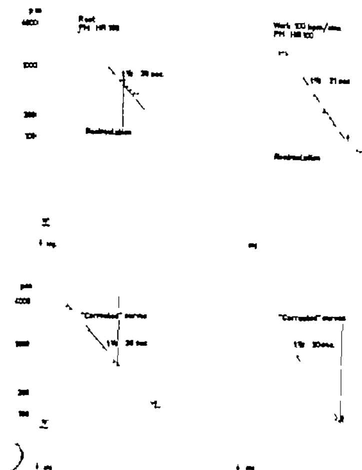


Fig 5 Clearance curves at rest and during physical work on one patient. The left side shows the clearance curve, recirculation curve and "corrected" curve at rest, and the right side shows the same during work. The recirculation level is lower during work and the influence on the curve configuration is less. The arrows indicates the approximate points of deviation of the curve.

effect of recirculation. The magnitude of the error expressed in per cent of half-time of the corrected curve is shown in Table VII. At rest there is an error of 4.5% and at work and during hyper ventilation the error is also less than 5%.

DISCUSSION

The deviation of myocardial isotope clearance curves from the ideal monoexponential function has been explained in various ways. Klein et al. (8) used selectively injected Krypton 85 in the coronary vessels and precordial counting. They considered this deviation secondary to isotope activity in fat and muscle tissue around the heart. However Shaw et al. (12) demonstrated that the same deviation of the curve could be found in isolated heart preparations and concluded that extracardiac structures could not be responsible. Bassingthwaighte et al. (1) also observed terminal deviation of clearance curves in isolated and open chest dog hearts. These authors concluded that variation of cardiac partitition coefficients (muscle and fat) probably explained the configuration.

The influence of the recirculation on myocardial clearance curves has to our knowledge not been previously explored. Holmberg et al. (6) showed that Xenon, injected in the descending aorta, resulted in an increase of activity in coronary sinus blood which must be explained by recirculation of isotope through lungs to left atrium and then through the coronary circulation.

Xenon and Krypton have been considered ideal myocardial indicators because of negligible recirculation to the left heart after passage through the pulmonary capillaries. Chidsey et al. (2) have shown, however that if Krypton 85 is venously infused continuously during ten minutes, 10–15% passes over to the arterial circulation. Presumably the same result could be expected for Xenon, which has similar physical properties. When Xenon or Krypton is injected on the arterial side,

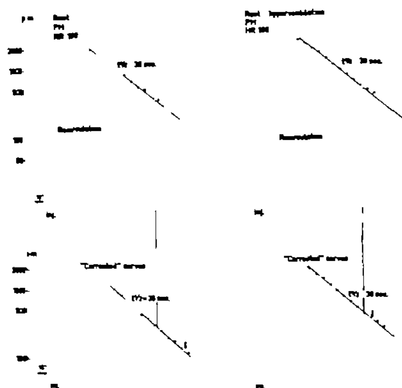


Fig. 6. Clearance curves at rest during normal ventilation and hyperventilation in one patient. The left side shows the clearance curve, recirculation curve and "corrected" curve with normal respiration, and the right side shows the same during hyperventilation. The recirculation activity is lower during hyperventilation, and the influence on the curve configuration is less. The arrows indicate the approximate points of deviation of the curve.

return to the pulmonary artery occurs over an extended period similarly to a continuous intravenous infusion, and thus a considerable recirculation through the lungs could be expected even with arterial injection. The present investigation reports the magnitude of this recirculation and its influence on the myocardial clearance curve recorded from the coronary sinus blood. The demonstrated pattern of recirculation activity with late appearance, slow increase and decrease most likely reflects the activity variation in pulmonary artery blood. This in turn is explained by the slow return of Xenon to the central circulation from different peripheral tissues with different partitition coefficients and flows. Isotope activity in pulmonay artery blood thus represents the sum of a number of clearance curves.

No particular attempts have been made to analyze the mechanism of recirculation through the lungs. However there is one special feature of the pulmonary circulation that could explain the recirculation phenomenon and also the fact

Table V *Recirculation at rest and during hyperventilation*

R = rest and spontaneous ventilation.
H = rest and hyperventilation.

Pat.	Condition	Deviation of clearance curve at of max. activity	Max. activity recirculation / Max. activity clearance curve (%)	$t_{1/2}$ recirculation
30	R	32	4 4	600
	H	16	1.3	?
31	R	23	4.8	243
	H	16	2.0	165
32	R	21	4.7	156
	H	9	2.3	85

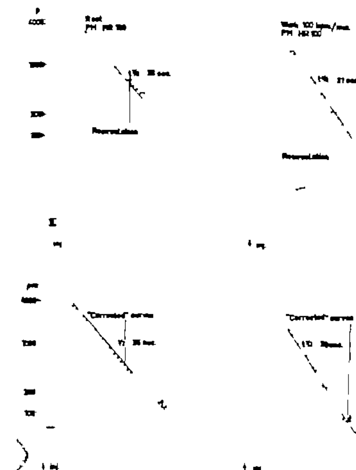


Fig 5 Clearance curves at rest and during physical work on one patient. The left side shows the clearance curve, recirculation curve and "corrected" curve at rest, and the right side shows the same during work. The recirculation level is lower during work and the influence on the *curve configuration* is less. The arrows indicate the approximate points of deviation of the curve.

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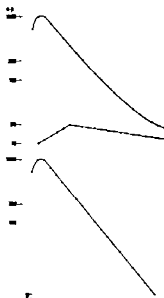


Fig 8 A constructed example showing the influence of recirculation on the clearance curve. T the monoexponential function curve at the bottom is added an activity curve of the same magnitude and shape as the recirculation activity measured in patients. The resulting curve at top shows marked deviation in spite of low recirculating activity level, in this case 2% of the maximal activity in the monoexponential function curve.

crease the shunting effect. Hyperventilation or increased ventilation during work are shown to decrease the atelectases and hence the shunting (4). The investigations on our patients take three to four hours, and it seems likely that small atelectases could develop during this period, with the consequences mentioned. Consistent with this hypothesis the recirculation level did decrease in all experiments with physical work and hyperventilation compared to the levels at rest.

Recirculation affects the clearance curve measurably despite low levels of recirculation activity This is illustrated in Fig. 8 with a constructed example. To a monoexponential straight line curve is added an activity of the same magnitude and shape as the recirculation activity measured in patients, and the resulting curve has a marked deviation.

The error imposed by recirculation if the initial slope is used for calculation is, however fairly small. In the group with a high coronary flow and recirculation still at a high level, the error averaged 4.5% This error should be lower at rest with spontaneous heart rate and during physical work. The studies during work support this view

The early and marked deviation of the clearance curve observed in the pacemaker-driven group might also be explained by the recirculation phenomenon. In group I work-induced heart rate increases are associated with increased ventilation. These factors lower the recirculation activity while they increase the coronary flow In group II the pacemaker-induced increase in heart rate also results in an increasing steepness of the clearance curve but the recirculation activity level is unchanged, because in these cases hyperventilation did not accompany the increased coronary flow An increased deviation of the curve then results. In these pacemaker patients even curves with a marked deviation approximate monoexponential functions after subtraction of the recirculation activity

Although recirculation is one major factor explaining the deviation of the myocardial clearance curve, there are other factors shown experimentally to be of importance. In several but not all patients in this series, even the corrected curve, where the influence of recirculation has been eliminated, has shown some deviation of the final part of the curve. This indicates that even in man there are other factors operating. However a further analysis of these factors has been beyond the purpose of this study

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THE FIDELITY OF A SAMPLING–RECORDING SYSTEM USED FOR MYOCARDIAL CLEARANCE CURVES WITH XENON 133 AS AN INDICATOR

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Abstract. The fidelity of sampling–recording system for recording of Xenon 133 myocardial clearance curves was tested in flow model in which monoexponential clearance curves could be produced. The sampling system did not induce any significant distortion of the clearance curves in spite of the low sampling speeds used, 5–25 ml per minute. This accurate response was attributed to details in the construction, which favored turbulence in the system.

Regional blood flow measurements by a clearance technique could include systematic errors when unsatisfactory sampling–recording systems are used which induce distortion of the clearance curve.

In a series of experiments myocardial blood flow has been recorded in man by a clearance method using Xenon 133 as an indicator and continuous recording of radioactivity in blood from coronary sinus (3). The blood was continuously sucked through a catheter system including a special cuvette, around which a well shaped scintillation detector recorded the isotope activity.

The aim of this study was to test the fidelity of the sampling–recording system used and to quantify the degree of distortion of the clearance curves caused by this system. Special attention was paid to the downslope of the curve.

METHOD AND PROCEDURE

A flow model of non-recirculating type was constructed as shown in Fig. 1. It consisted of an upper fluid container ith constant water level to guarantee constant driving pressure through the flow system. Connected to this by large-diameter tubing was mixing chamber with two motor-driven mixing propellers. The volume of the mixing chamber was 1 000 ml. At the inflow of the chamber was an injection needle with stopcock for the injection of indicator. The out-flow side of the mixing chamber was connected by large-diameter tubing to flowmeter and thence to lower fluid container which also had constant water level. On the out-flow side of the flowmeter was constrictor on the tubing for regulation of flow. Under the mixing chamber scintillation detector was placed with 2" NaJ crystal and cone-shaped lead collimator recording isotope activity from the mixing chamber only.

After the constrictor on the out-flow tubing was connection for the recording system to be tested. The system consisted of an Eppendorf catheter no. 8 with the tip of the catheter placed 10 cm in retrograde direction from the entrance. The catheter was connected to the cuvette, glass spiral with an internal volume of 1.4 ml, and this in turn was connected via another catheter to motor driven syringe for continuous suction at variable speed. The suction speeds used in these experiments ere 25 and 5 ml/min. The volume of the sampling system up to the end of the glass spiral was 3.3 ml. The glass spiral was placed in ell detector consisting of a 2" NaJ crystal connected to photomultiplier. The impulses from both detectors are fed into scaler with pulse height analyzer (Nocab, Sweden). The activity was recorded on tape hich allowed quantitative recording ithout inertia. The activity was later analysed via semiautomatic printer with two alternating counters hich, during play back of the tape, counted and printed the activity for predetermined time intervals. This activity was then plotted against time on semilog paper. The sampling and recording system was identically built and used as in the in vivo experiments.

In the in vivo experiments an aqueous solution of radioactive Xenon 133 was used as indicator. In these model experiments, though, the same indicator could not be used because the mixing chamber and other parts had to be manufactured from acrylic, substance to hich Xenon has high affinity. Therefore J^{131}-albumin was chosen instead as indicator. This substance has been reported to adhere to glass surfaces, which could possibly influence the recording of the clearance curve by continuously in-

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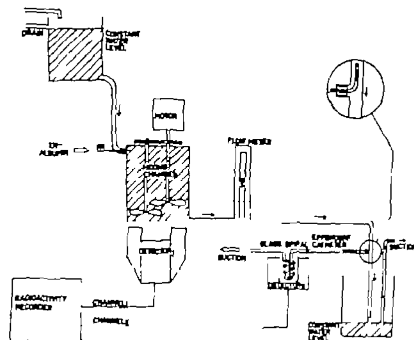


Fig 1 Schematic drawing of flow model with sampling–recording system.

creasing background activity during the recording period. T exclude significant adhesion the following experiment as made. The piral was connected to both a container with J^{131}-albumin solution and container with water via a three-way stopcock. Alternatingly water J^{131}-albumin, and water again, was sucked through the spiral at speed of 12 ml min. The activity was recorded by the well detector The level of background activity before and after exposure of the spiral to high J^{131}-albumin concentrations was used for evaluation of adhesivity

Fo the use of the flow model in testing clearance curves instantaneous mixing of the indicator is essential. To test this, the indicator was injected into the mixing chamber with and without propeller stirring. In all experiments the activity in the mixing chamber was recorded by the attached detector. The shape f the activity curve and the time it took to reach a stable activity level reflected speed and effectiveness of mixing.

T evaluate the distortion f clearance curves in the sampling system, the flow model and the complete sampling–recording system as shown in Fig. 1 were used. The flow through the system was kept at constant rate with the clamp in most experiments 1 000 ml/min, and in a few cases 2 000 ml/min. Because of mechanical inefficiency the flow varied $\pm 2\%$ according to flowmeter readings. After 1 min recording of background, J^{131} albumin was momentarily injected. The amount of indicator used gave maximum activity of approximately 5 000 cpm in the detector of the mixing chamber, which reached in 6 000 cpm in the well detector

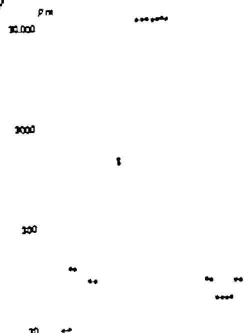


Fig 2 Isotope activity curve obtained when sampling-recording system exposed to square wave of J^{131}-albumin solution. Suction speed through sampling system 12 ml/min.

RESULTS

I Ten experiments were made to measure J^{131}-albumin adhesion. The result from one of them is shown in Fig. 2. The glass spiral was exposed for 30 sec to a J^{131}-albumin concentration five times higher than that used in the following flow studies. Background activity both before and after the period of exposure was less than 0.5% of max. activity and there was no measurable increase.

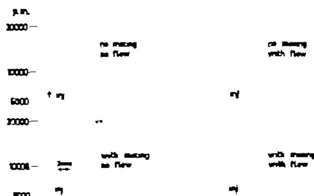


Fig. 3. Activity recorded from detector attached to mixing chamber. Upper part: activity pattern without mixing function; to the left without, and to the right with flow through flow model. Lower part: activity pattern with mixing function; to the left without, and to the right with flow through flow model.

The results were the same in all experiments. Adhesion of I^{131}-albumin to the glass spiral was thus in these experiments without measurable influence on the curves.

II. The importance of good mixing is illustrated in Fig. 3. Without mixing, the activity recorded from the detector under the bottom of the mixing chamber was slowly rising. With propellers functioning, there was almost instantaneously a stable activity level. With the asymmetric position of the detector and no flow through the mixing chamber an increasing activity must be interpreted as diffusion of indicator from the site of injection towards the detector, i.e. the activity curve reflects a mixing procedure. With propeller mixing the activity reaches 95% of maximum in less than 2 sec, while it takes 35 sec to reach the same value without mixing. The same principal difference in mixing pattern between tests with and without propeller function was obtained when the flow passed through the mixing chamber. This experiment showed that mixing with propellers is so efficient that the clearance curve from the mixing chamber could be regarded as representing a true monoexponential function.

III. The results of the clearance experiments are presented in Table I. The half-life ($t\frac{1}{2}$) for the clearance curves from the spiral and the mixing chamber are compared for each experiment. In this comparison the clearance curve from the

Table I. *Results of clearance experiments*

Expt. no.	Flow in sampling system (ml/min)	Flow in flow model (ml/min)	$t\frac{1}{2}$ of clearance curves from spiral (sec)	$t\frac{1}{2}$ of clearance curves from mixing chamber (sec)	Difference spiral/mixing chamber (sec)
1	25	1 000	51	51	0
2	25	1 000	51	51	0
3	5	1 000	49	49	0
4	25	1 000	50	50	0
5	5	1 000	49	49	0
6	25	1 000	51	51	0
7	5	1 000	49	49	0
8	25	1 000	51	51	0
9	5	1 000	52	52	0
10	25	1 000	52	52	0
11	25	1 000	52	52	0
12	5	1 000	50	50	0
13	5	1 000	52	52	0
14	5	1 000	53	53	0
15	25	2 000	26	27	−1
16	25	2 000	28	28	0
17	25	2 000	27	27	0

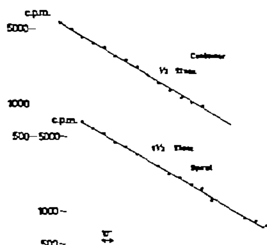


Fig 4 Simultaneously recorded clearance curves from cuvette (spiral) and mixing chamber (container) in one experiment.

mixing chamber was used as reference, represent ing the "true" clearance curve. One example of clearance curves is given in Fig. 4 In 14 of the 17 experiments the system flow was 1 000 ml/min, and in these experiments the flow speed through the sampling system was alternated between 5 and 25 ml/min. In all these experiments the *t* ½ of the two clearance curves was identical. In three experiments the system flow was 2 000 ml, and the flow speed through the sampling system 25 ml/min. In one case there was a difference between the two simultaneous clearance curves of 1 sec (2 %), in the two others the *t* ½ values were identical. The *t* ½ varied a little from experiment to experiment, from 49 sec to 53 sec, reflecting the inefficiency of the flow adjusting mechanism.

DISCUSSION

Flow measurements by clearance technique include in most cases, sampling through catheters. Such a sampling technique always causes a longitudinal dispersion of the indicator and a distortion of the clearance curve when the flow is laminar The magnitude of the error introduced is difficult to calculate because the sampling systems are more complicated than the simple straight tubes on which known formulas may be applied (2, 7 8, 9). Therefore, for practical pur poses each sampling system must be separately tested for distortion (6).

The system tested was designed exclusively for clearance studies on heart muscle, only the downslope of the clearance curve being used for calculations. Therefore only the distortion of this variable has been investigated. For practical reasons all the tests were performed with water However it has been shown by others that in similar sampling systems with controlled flow there is no significant difference in distortion between trials with water and with blood (5).

In our experiments there was no significant distortion of the downslope of the clearance curve when the flow speeds in the sampling system varied from 5 to 25 ml/min.

The dimensions of the sampling system and the flow rate are factors that influence the distortion (2, 4), and formulas have been deduced empirically to predict the degree of distortion for dif ferent variables based on the volume flow ratio (5). These formulas do not seem to hold true for all systems and other factors must be taken into consideration, e.g. geometry of the catheter type of cuvette etc. (1).

Considering the slow sampling speeds used, our results are more favorable than would have been expected from these formulas. These differences in results could be explained by differences in sampling system. In contrast to others we have used a sampling catheter with small side holes and a spiral-shaped cuvette, and both these fac tors favor turbulence (1 6) which diminishes the longitudinal dispersion of the indicator

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HYPEROSTOSIS CORTICALIS GENERALISATA

Eight New Cases

F. S. P. van Buchem

Abstract Eight new cases of hyperostosis corticalis generalisata are described. All showed the same typical localization of the increased bone formation. In three children (ranging in age between seven and ten years) this was already clearly visible, even though not so marked as in the adults. The clinical picture was suggested on a photo of the skull taken at the age of two. The disease may therefore start at a very early age, and then may be correlated with the often occurring facial paralysis, which in the beginning was not rarely transient. Early operation is certainly indicated in these patients. All eight patients proved to be related to each other. The pedigree chart is indicative of the autosomal recessive nature of the disease. In six adult patients suffering from this disease the iso-enzyme pattern of alkaline phosphatase was examined. The bone fraction was present in all cases, although in two cases alkaline phosphatase was not increased.

Hyperostosis corticalis generalisata is a hereditary systemic disease of the skeleton. Based on studies in seven adult patients (1, 2, 3) I concluded that an excessive formation of normal bone tissue was involved, and that the clinical picture differed from the systemic skeletal diseases described so far, which was confirmed by Hinkel (4) and Rubin (5). The present paper is a report of the findings in eight new patients suffering from this disease, among whom three children.

CASE REPORTS

Case 1

An unmarried woman, born in 1915. Since her birth the parents had suspected that her vision was poor. Menarche started at the age of fifteen. At fourteen she developed a bilateral peripheral facial paralysis. At thirty-six a slowly progressing paralysis of her legs developed, and she has not been able to walk since 1955. At forty-two she was blind as a result of amaurosis congenita (Leber) (7). Her hearing was poor, especially in the right ear. The chin was widened, angular and markedly thickened, no prognathism. The auditory disturbance was based on bilateral perception deafness. Her height was 166 cm, weight 66.5 kg. Blood pressure 140/90. Heart and lungs were normal, liver and spleen not palpable. A firm resistance, the size of a nut, was palpable in the right hemithyroid. The ECG was normal. Haemoglobin 95%, erythrocytes 4 830 000, leucocytes 5 300, eosinophils 3%, staff forms 2%, segmented forms 60%, lymphocytes 31%, monocytes 4%. Sedimentation rate after 1 h 5 mm, syphilis reactions negative, serum cholesterol 257 mg%, acid phosphatase 3.4 U. Protein spectrum: total protein 8.1 g%, albumin 47.5%, α_1-globulin 5.6%, α_2-globulin 10.4%, β-globulin 12%, γ-globulin 24.5% (Table I). The 24-hour excretion of 17-ketosteroids was 2.3 mg, of hydroxyproline 49.8 mg.

The EEG showed a low voltage with very little alpha rhythm.

In 1968 she was seriously ill due to a bulbar paralysis caused by a circulatory insufficiency of the basilar system (Dr J. Dijkstra). The EEG showed a serious regulatory disturbance with changes of the temporodiencephalic system. The patient recovered well from this.

The still present neurological abnormalities will be described extensively in another publication.

Finally the patient has a third hereditary disease: alkaptonuria. Her brother is also blind due to the amaurosis congenita (Leber). A sister is healthy.

Case 2

A man, born in 1921. He had no complaints and was still able to do his heavy physical work. He has had a bilateral peripheral facial paralysis for many years. The lower jaw was widened, angular, markedly thickened with normal implantation of the teeth, no prognathism. Serum calcium and phosphate were normal, as also the alkaline phosphatase (Table I). The youngest of his six children, born in 1961, had multiple sutural synostoses of the skull with syndactylia of the ring fingers with the little fingers, of the index fingers with the middle fingers, and of the four lateral toes, but no syndactylia osseа

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Table I. *Laboratory data*

Cases	1	2	3	4	5	6	7	8
Age (y)	52	47	37	22	19	10	8	7
Sex	♀	♂	♀	♂	♀	♂	♀	♀
Serum calcium (mg %)	9.8	10.8	9	9.1	10.3	9.9	10.1	10.3
Serum phosphate (mg %)	4	3.6	4	3.6	3.8	5.1	5.8	5.8
Alkaline phosphatase (K.A. units)	14.1	10.1	12	21.7	<12	51.2	45	29
Facial paralysis	R + L	R + L	R + L	R + L	R + L	L	R	R

(syndromes of Apert and Crouzon). Heart and lungs were normal, liver and spleen not palpable. In this girl the serum calcium was 10.6 mg% phosphate 5.2 mg% alkaline phosphatase 15.2 King-Armstrong units.

Case 3

A married woman, born in 1932, developed in her youth right-sided peripheral facial paralysis, but had no other complaints. In 1958 she gave birth to healthy child, following which menstruation did not return. In 1959 sh had severe headaches, later associated with vomiting. When she sought medical treatment for this, the large skull, the widened and thickened mandible and the thickened clavicles were noticed (Dr W van Bork). She had an upward nystagmus, with an anticlockwise rotatory component, and strong nystagmus to right and left. Otherwise medical and neurological examination revealed no abnormalities. Vision and ocular fundus were normal. Serum cholesterol 171 mg% urea 21 mg% magnesium 1.8 mEq/l. Leucocytes 5 100, eosinophils 4% staff forms 6% segmented forms 64% lymphocytes 24% monocytes 2%. The urine was normal. The 17-ketosteroid excretion was 14.1 mg/24 h. The ECG was normal. The EEG showed bradyrhythmic curve as seen in disturbance of the brain stem function. At the age of 28 she developed a bilateral peripheral facial paralysis.

In 1968 she still had pains on the left side over the ear on the left of the neck and in the left arm, radiating to the fingers. Physical examination showed no changes of heart and lungs. Liver and spleen were not palpable. Blood pressure 136/98. The thyroid was not enlarged. The force of the left arm and left hand was clearly less than in right arm and hand.

Case 4

A man, born in 1946, had no complaints and was well able to do his heavy work as sea fisherman. In 1949 he had had a transient right-sided facial paralysis. He had been rejected for military service because of insufficient auditory acuity and disharmonious personality structure in 1965. In 1966 he was treated for commotio cerebri (accident on moped). Then the marked osteosclerosis of the base and roof of the skull and of the ribs and clavicles was visible. In 1967 he was under treatment for coma caused by a motor-car accident, in which the diagnosis of cerebral contusion was made. (Dr J Dijkstra). The EEG was disturbed diffusely without lateralization phenomena. He had no choked discs, the fundus oculi was normal. No abnormalities were found in the general and neurological examination, blood pressure 140/80.

In 1968 he had bilateral facial paralysis, markedly thickened lower jaw no prognathism. His hearing was poor in the right ear. Besides the marked thickening and sclerosis of the base and roof of the skull, the diaphyseal cortex of the long bones was thickened. The serum phosphatase was increased (Table I). This patient is a brother of case 5

Case 5

A woman, born in 1949 had a left-sided facial paralysis at the age of two, and right-sided at the age of five. Otherwise she had not been ill. Menstruation had been regular since the age of thirteen. She had been troubled by mild headaches during the past three years. She was sometimes dizzy when suddenly getting up. Occasionally she had more pain especially on the left side of the head, and at the same time numb feeling in the left half of the face and left side of the tongue, sometimes with nausea and occasional vomiting.

At the age of thirteen the wide angular, thickened mandible was already visible (Fig. 1). Heart and lungs were normal at the general physical examination. Liver and spleen were not enlarged. Nor was the thyroid. Blood pressure was 145/85, height 171 cm, weight 69 kg. She had bilateral peripheral facial paralysis, otherwise no



Fig 1 Case 5 age 13 years. Thickening of the chin.

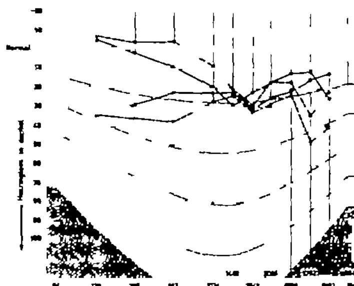


Fig. 2. Case 6, age 10 years, audiogram. Loss of air conduction in the bass zone and of bone conduction in the descant zone. O air conduction; ● bone conduction — right ear left ear

neurological abnormalities. The EEG did not show any pathological changes. Otological examination revealed no disturbances of hearing or equilibrium. She had an orthostatic vertigo.

Case 6

A boy born in 1958 had left-sided peripheral paralysis, which recovered spontaneously at the age of two, one week after smallpox vaccination. At the age of eight he again developed left-sided facial paralysis. As this did not recover spontaneously the neurologist (Dr J. Dijkstra) advised liberation of the nerve. Owing to circumstances this was postponed and, when the operation was performed after about year recovery did not occur. The skull photo taken at the age of ten clearly showed the thickening of base and roof of the skull. The petrous bone was especially noteworthy which was already visible on the skull photo of 1960 when the patient was two years old. The frontal, sphenoid and maxillary sinuses were well developed.

In 1968 he had no complaints other than occasional headaches. He was well-built boy height 143 cm, weight 32.5 kg. The thyroid was not palpable. Blood pressure 110/70. Operation scar behind the left ear, peripheral facial paralysis on the left, on the right only of the frontal branch. The mandible was somewhat widened and the edge thickened. Heart and lungs were normal, liver and spleen not palpable.

The audiogram (Dr G. van Waning Bolt) (Fig. 2) showed air conduction disturbance in the bass zone, and bone conduction disturbance in the descant zone.

Urine: protein, glucose and urobilin negative, specific gravity 1.020, 4-hours hydroxyproline excretion 56.4 mg. Haemoglobin 81%, erythrocytes 4 360 000, leucocytes 6 600, eosinophils 6%, segmented forms 52%, lymphocytes 37%, monocytes 5%, sedimentation after 1 h 3 mm, serum acid phosphatase 3.4 units (Table I).

The patient is brother of patients 7 and 8.

Case 7

A girl, born in 1960, had not been ill until, at the age of seven, she developed right-sided facial paralysis. Abnormalities of the skull were found, in particular thickening of base and roof of the skull. The Stenvers photo of the petrous bone clearly demonstrated sclerosis, the canalis Internus was narrowed. Operation (Dr G. van Waning Bolt) revealed very thick mastoid cortex, the facial nerve was surrounded by massive bone and was as thin as horse-hair. The neural function recovered after few weeks. The audiogram (Dr G. van Waning Bolt) showed slight loss of air conduction in the bass zone. In 1968 she was symptom-free. The general physical examination showed operation scar behind the right ear, paresis of the frontal branch of the right facial nerve, blood pressure 116/60, height 127 cm and weight 24 kg. The thyroid was not palpable, heart and lungs were normal, liver and spleen not palpable. The central part and the two arcs of the lower jaw seemed somewhat thickened. Urine protein, glucose and urobilin negative, Sulkowitz' reaction negative, 4-hours hydroxyprolline excretion 33.2 mg, serum acid phosphatase 4.4 units (Table I). Roentgenologically the skeleton already showed thickening of roof and base of the skull with marked pneumatization, striking sclerosis of the petrous bone (Stenvers photo), the mandibular cortex was thickened; the diaphyseal cortex of humerus, femur, tibia and fibula was thickened too, and indications of thickening were seen in ulna and radius. The spinal processes of the vertebral column were hyperostotic.

Case 8

A girl, born in 1961 developed at the age of two right facial paralysis, which recovered spontaneously. This returned and occurred for the third time at the age of five, but now spontaneous recovery did not occur. In view of the skull abnormalities found, operation was decided on



Fig. 3 Case 1 Thickening of the skull and mandible.

(Dr O. van Wering Bolt). Here the findings were the same as in her sister (patient 7). The pathologist found only compact bony tissue with cell-rich marrow without any particular abnormalities in the pieces of mastoid cortex. The audiogram was still practically normal.

*In 1968 she had no complaints. General physical ex*amination showed, operation scar behind the right ear paresis of the frontal branch of the right facial nerve, blood pressure 110/60, height 119 cm, weight 22.1 kg. The thyroid was not palpable. Heart and lungs were normal, liver and spleen not palpable. Urine: protein, glucose and urobilin negative, Sulkowitz' reaction negative, 24-hours hydroxyproline excretion 38.5 mg, serum acid phosphatase 4.9 units (Table I). Even as early as the age of seven, the X-ray showed thickening and hyperostosis of base and roof of the skull, especially of the petrous bone with, for her early age, a particular development of the frontal, sphenoid and maxillary sinuses, further thickened ribs and clavicles, high calcium content of the spinous processes of the vertebrae thickened diaphyseal cortex of humerus, ulna and radius.

RESULTS AND DISCUSSION

Clinical manifestations

The age of the five adult patients varied between 19 and 52 years. The three children were 10, 8 and 7 years old. Three patients were males and five females. Taken as a whole, the two sexes were therefore practically equally represented among our 15 patients in the two series.

In the present study too, all adult patients showed the *wide, angular and markedly thickened* lower jaw which is so characteristic that in itself, it may suggest the diagnosis. One might think



Fig. 4 Case 8, age 7 years. Well developed frontal and sphenoid sinuses. Thickening of the skull.

of acromegaly but these patients have no prognathism, the position of the teeth is normal, tongue and nose are not enlarged, and, also in other respects, there are no indications of acromegaly. The peculiar chin was seen in one of our patients (case 5) even at the early age of thirteen (Fig. 1). Among the children the thickened mandible was clearly palpable only in the 10-year-old boy (case 6). In adult patients the thickened clavicles are also palpable. The long bones of the extremities are not thickened. The affected bones are not painful to pressure. The patients are undisturbed in their movements, as the joints and muscles are normal. A firm swelling, the size of a nut, was palpable in the right half of the thyroid in one of the patients (case 1).

In this series facial paralysis was found in all cases, bilateral in all adults, and unilateral in the three children. The three children and cases 4 and 5 suffered from paralysis already during the first years of life: in the beginning this was transient. The boy (case 6) had a left-sided paralysis at the age of two: it recovered spontaneously but recurred at the age of eight, then it was permanent, even though an operation was performed later on. His sister (case 8) suffered from paralysis for the third time at the age of five. The second sister (case 7) had paralysis when seven years old. Both girls were operated on (Dr G. van Waning Bolt) by liberation of the facial nerve. The bony canal was very narrow and the facial nerve thin as a horse-hair. Nevertheless recovery set in some weeks post-operatively: this has been maintained up to the time of writing, and 2 /₃ years later respectively. Only the frontal branch of the facial nerve did not recover in both of them. The thickening and sclerotic changes of the roof and base of the skull, especially of the os petrosum, were visible even at the age when they were subjected to surgery in the boy at the age of two. The cortex of the mastoid was very thick, and macroscopically compact bony tissue with cell-rich marrow was seen, without other particular features. Witkop (9), too, found facial paralysis in one patient at the age of 4–5 years. The initially transient facial paralysis may be a result of an already beginning compression, which makes the facial nerve more vulnerable.

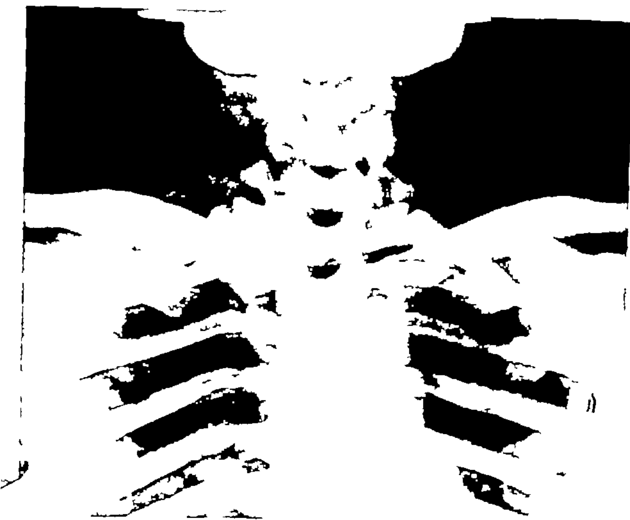


Fig. 5 Case 2. Sclerosis of the ribs and clavicles.

More or less marked hearing disturbances as well were often present in this series of patients. An audiogram was taken of the three children (Dr G. van Waning Bolt). It was still normal in the youngest child (case 8) but showed a loss of air conduction in the bass zone in the two other children (cases 6 and 7), most marked in the oldest (case 6), who also had some loss of bone conduction in the descant zone (Fig. 2).

Both the facial paralysis and the loss of bone conduction can be explained by the narrowing of the foramina of the cerebral nerves in the base of the skull however this narrowing need not always be so extreme that these abnormalities arise. This also holds for the foramen opticum. Two patients from the first series initially showed, at the age of 28 and 37 respectively choked discs, and ten years later both had bilateral atrophy of the optic nerves.

Case 1 of this series also suffered from serious visual disturbances as a result of congenital amaurosis (Leber)· this disease was found in thirteen members of her family also in her brother (7), who, however had no hyperostosis.

Laboratory investigations

Just as in the first series of patients, the serum calcium and inorganic phosphate were normal (Table I). Here we should not forget that, normally in subjects below the age of twelve, the inorganic phosphate is somewhat higher than in adults. The serum alkaline phosphatase was clearly raised in two adults (cases 1 and 4) and in the three children (cases 6, 7 and 8), in one bordering

on normal (case 3) and in two (cases 2 and 5) normal. It had been elevated in six of the seven patients of the first series. In recent years several cases of this disease have been described, some of whom had an elevated alkaline phosphatase but most of them not (6, 8, 10). This was also the case in Witkop's (9) patients.

In one of the patients of our first series (ref 3 case 6), who was 23 years old at the time, the alkaline phosphatase had been markedly raised (26.7 King Armstrong units), but seven years later it was 3.6 Bessey units. It was markedly increased in case 4 of this series, but not in his sister case 5 who was younger. It may therefore vary strongly in the same patient, which may be associated with the greater or lesser activity of the process at this particular moment.

Dr H. A. Zondag analysed the iso-enzyme pattern of the serum alkaline phosphatase, using agar gel electrophoresis in six patients suffering from this disease (ref 3 cases 5 and 7 and cases 1 3 4 and 5 of this series). In normal adults the

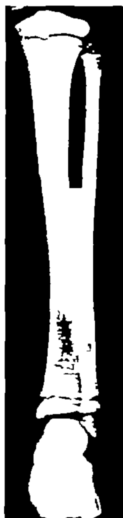


Fig. 7 Case 6, age 10 years. Thickening of the diaphyseal cortex of tibia and fibula.

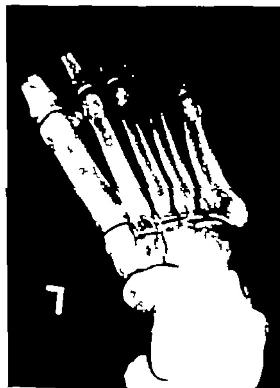


Fig. 6. Case 3. Thickening of the diaphyseal cortex of the metatarsals.

bone fraction is absent. In the patients who were older than 20 at the time, the bone fraction was present, although in two cases, 3 and 5 the serum alkaline phosphatase amounted only to, respectively [illegible]3 and [illegible]8 Bessey units at that time. This is very remarkable finding.

Russell et al (6) found normal 24-hour urinary excretion rate and normal renal clearance of calcium, phosphorus and creatinine. No abnormalities of the urine or of the leucocyte picture have been found in any cases known up to now but case 1 of this series had also alkaptonuria.

X-ray investigation

All patients again showed the characteristic symmetrical abnormalities of the skeleton. The trabe-



Fig. 8 Case 6, age 10 years. Thickening of the skull and sclerosis of the petrous bone.

culae of compacta and spongiosa had increased uniformly in thickness with normal arrangement of the spongiosal trabeculae. Base and roof of the skull were markedly thickened in all of them, but all sinuses were always clearly pneumatized, with thickened walls and a normal sella turcica (Fig. 3, case 1). In the youngest children (cases 7 and 8) the frontal and sphenoid sinuses were remarkably well developed for their age (Fig. 4 case 8, age seven).

The clavicles and ribs were hypertrophic with a reduction of the medullary cavity as a result of thickening of the compacta (Fig. 5 case 2).

The diaphyseal cortex of the long bones, metacarpals and metatarsals (Fig. 6, case 3) was thickened without increase of the diameter. In the present series again, the vertebral bodies showed little abnormality The spinal processes, however showed marked hyperostosis as described before, also in the children. Excrescences are often observed in older patients.

The roentgenograms of the children showed already the same changes of the long bones (Fig. 7 case 6), the hyperostosis of the clavicles and the ribs, and especially of the roof and the base of the skull, in particular the os petrosum (Fig. 4 case 8 and Fig. 8, case 6). In case 6 the osteosclerosis and the thickening of base and roof of the skull were already visible at the age of two (Fig. 9).

The strong development and thickening of the mandibles, so striking in all adults, was not yet outstanding in the youngest children (cases 7 and 8), even though the photo of patient 6 showed a definite thickening of the mandibular cortex.

These observations in children prove that this disease may develop as early as the first years of life and may then become manifest especially in the skull and particularly in the os petrosum.

Pathology

In the previous publications (1 3) an extensive report was given of the autopsy of one of our patients. Witkop (9) informed us that also in one of his patients autopsy had been carried out, and that his autopsy findings were essentially the same as in our case. Microscopic examination of the iliac crest and the lower jaw of case 1 also showed that the compacta consisted of mature lamellar bone with narrow haversian canals, and here and there some osteoblastic activity The bone marrow was normal. The course of the fibres was



Fig. 9. Case 6, age years. Thickening of the skull.

shown to be normal in polarized light. The biopsies suggested endosteal and periosteal formation of mature lamellar bone especially in the cortex.

Aetiology and pathogenesis

In the previous series there were already indications that the disease is hereditary. This became clearly manifest in this new series, all living at Urk. As found by Schappert-Kimmijser et al. (7) in their study of amaurosis congenita (Leber), the demographic history of the population of this former island Urk affords an ideal object of study for population genetics. In 1637 only 151 of the 300 inhabitants survived the plague. As, according to these authors, many of the marriages were endogamous up to the year 1941 when the part of the Zuyderzee in which Urk was situated was reclaimed, and consequently Urk was no longer an island, the present population may be regarded as descendants of about 75 marriages in the seventeenth century.

All the eight patients of this series proved to be related to each other. The pedigree chart (Fig. 10) shows that the eight patients are descendants from three families (Ha.–Kie., L. Ro.–G. D. and A. Ras.–Kl.). Both the mother and the father of case 1 are descendants from Ha.–Kie. Her mother is also a descendant from L. Ro.–G. D. and her father from A. Ras.–Kl.

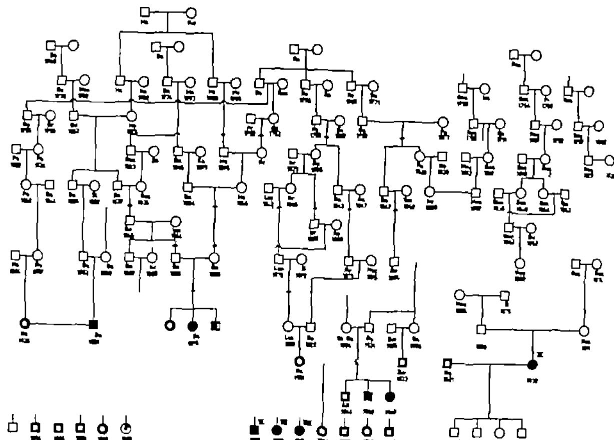
The father of case 2 is descendant from Ha.–Kie and his mother from A. Ras.–Kl. A grandfather of case 2 is brother of great-grandfather of case 1.

The parents of case 3 are both descendants from A. Ras–Kl. the father also from L. Ro.–G. D.

The parents of cases 4 and 5 are both descendants from L. Ro.–G. D. and also both from A. Ras.–Kl. A grandfather is brother of the father of case 1.

The parents of cases 6, 7 and 8 are both descendants from L. Ro.–G. D., and the father also from A. Ras.–Kl. A grandmother of cases 6, 7 and 8 is a sister of the father of cases 4 and 5.

None of the parents or the children of the affected persons were affected. If we take the previous and the present series together both males



10 Pedigree chart of a family in a Dutch isolate including eight cases of hyperostosis corticalis generalisata.

□ male ○ female ■ ● affected; □ ○ examined, date, birth year

and females were about equally affected. From the above it seems justified to assume the autosomal recessive nature of the disease.

Recessive genes for abnormal traits can act only in homozygous persons. As the number of common ancestors increases, so does the probability of two recessives combining to form one abnormal homozygous person.

Case 1 was also suffering from alkaptonuria and amaurosis congenita (Leber). The other patients of this series had no alkaptonuria and no amaurosis congenita (Leber). A brother of patient 1 with amaurosis congenita (Leber) had no lesions of the skeleton.

The youngest daughter of case 2 showed the typical picture of a combination of Crouzon's dysostosis craniofacialis and Apert's acrocephalosyndactyly with the typical turricephalus with little developed occiput, widened distance between the orbits, shallow orbits, exophthalmus, deepened digital impressions, hypoplastic upper jaw syndactyly of the little finger with the ringfinger and of the index finger with the middle finger respectively. These, too, are hereditary diseases.

Russell et al. (6) found their patients during a periodic examination of the population in Hiroshima and Nagasaki, which was started in 1958. The two oldest patients, aged 44 and 39 sisters, had been exposed to the atomic bomb at a distance from the hypocentre greater than 5 000 metres. The remarkable feature was that both the mother (aged 44) and all her four children were among the patients (23–15 years). This certainly did not occur in the other known cases of this disease.

In the chromosome study of Turner and Kelly (8) a group of 45 X0 chromosomes was found besides the usual 46 XX chromosomes, and moreover a combination of chromosomes characterized by the loss of an X chromosome, and the loss or translocation of an F chromosome.

The chromosome study in three of our pa-

tients from the first series did not reveal any disturbances, nor in case 1 of the present series.

Differential diagnosis

In an earlier publication (3) we extensively discussed the differential diagnosis from other systemic diseases of the skeleton (marble bone disease of Albers-Schönberg, osteomyelosclerosis, diaphyseal dysplasia of Camurati-Engelmann, hyperostosis generalisata with pachydermia of Uehlinger) we therefore shall not revert to it here. We only wish to point out that Russell et al. (6) unjustifiedly in our view believe that one can speak of variants of this disease, because they did not find an elevated alkaline phosphatase in all patients, because four of the five patients were women, and because they did not observe facial paralysis and bony excrescences. These, however do not constitute essential differences from the clinical picture described by us, but can be explained on the basis of the duration and the greater or lesser activity and extension of the process. Moreover up till now the bone fraction of alkaline phosphatase was present even in cases without increase of serum alkaline phosphatase (see page 263).

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Table I. *Patients excluded from the trial, arranged according to reason for exclusion*

Reason for exclusion	No. of pats.
Duration of treatment less than 18 months	49
Temporary interruption of treatment	6
Low dosage because of cerebral hemorrhage	1
Incomplete data	1
Non-cooperation	1
No comparable control patient available	5
Total	63
Trial	97
Death from recurrence	160

as whole was compared. Since sudden death, as has been stated, is not always due to a recurrent myocardial infarction, the clinical recurrences and the sudden deaths were separately compared with the control patients, so that difference in correlation between the degree of anticoagulation and death in both groups should become evident. Because badly regulated patients are checked more frequently six of all thrombotest values obtained during the year of investigation were collected for each patient by taking the value of the first check made in

Table II. *Composition of the group*

II

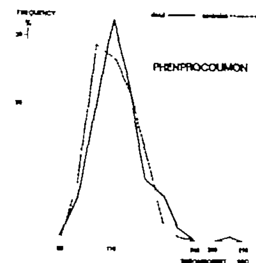
Age at death (y)	Sex ♂	♀
40–49	2	—
50–59	24	2
60–69	30	9
70 and older	23	7
Total	79	18

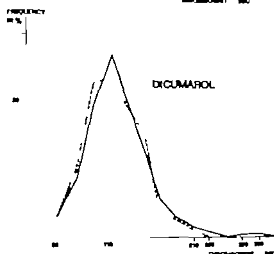
II*b*

Duration of treatment (y)	No. of pats
1½–2	13
2–4	27
4>	57

II

Drug	No. of pats.
Acenocumarine (Sintrom)	25
Dicumarol (Dicumol)	47
Ethyldicumarol (Tromexan)	1
Phenprocoumon (Marcoumar)	24





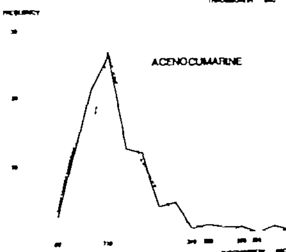


Fig. 1. The intensity of anticoagulation in the group of deceased patients and the control group, according to the drug used, taken from six thrombotests of each patient. The apices of all curves lie at approximately 110 sec.

Table III. *Mean of all thrombotest values in seconds in deceased group and control group*

The differences in these averages are not significant at 10% level, either per drug or for the three drugs combined. This was tested by means of the Student' *t*-test for paired observations

	Phen-procoumon	Dicumarol	Acenocou-marine	Total
Deceased patients	117.0	119.4	118 0	118.3
Controls	111 7	114.6	117.9	114.9
Difference	5.3	4.8	0 1	3.4

alternate months. The total number of thrombotests done and the amounts of anticoagulant administered are also recorded. For the calculations the patients were classified according to the anticoagulant used.

The various anticoagulants were used in all patients of the Service in the following proportion: phenprocoumon : dicumarol : acenocumarine – 5 7 8.1 5.5 The difference between this proportion in the whole group and in the group of examined patients (1.2 2.3 1.2) is not significant. The thrombotest values were calculated not in percentages, but in seconds, to avoid possible source of reading error; the various batches of the thrombotest used during the year of the investigation showed no appreciable differences in intensity

Table IV *Standard deviations of logarithms of thrombotest values*

– – average of each pat. d = deceased pats. = controls

	No. of pats.					
	Phen-procoumon		Dicumarol		Acenocou-marine	
S.D.	d		d		d	
0.02–0.05	5	5	3	10	2	4
0 06–0 09	11	13	18	13	7	5
0.10–0.13	6	6	9	12	7	6
0.14–0 17	1	–	13	9	3	6
0.18–0.21	–	–	1	2	5	1
0.22–0.25	1	–	2	1	1	3
0.26–0 29	–	–	1	–	–	–
Total	24	24	47	47	25	25
Median	0.08	0 07	0.11	0.10	0.12	0.12
	0 077		0 107		0.120	

are not significant, either per drug or for the three drugs combined (tested by means of the differences in the average of each pair).

The amount of anticoagulant used is also a measure of the intensity of treatment. For all the preparations these amounts were converted into mg phenprocoumon. The distribution of the amounts is given in Fig. 2, from which it can be seen that these differences, too, are small.

RESULTS

I. *Intensity*

The anticoagulation intensity in both groups (deceased and control) according to the drug used is shown in Fig. 1 The apices of all curves lie at approximately 110 sec.

The average thrombotest values are given in Table III. The differences between these averages

II. *Variability*

As measure of variability the standard deviation was calculated from the average of the logarithms of the thrombotest values of each patient. The results are shown in Table IV For each kind of anticoagulant the standard deviations in the two



Fig. 2. Quantity of drug used. The amounts for all the preparations were converted into mg phenprocoumon.

No. of thrombotests	Phenprocoumon Deceased pats	Phenprocoumon Controls	Dicumarol Deceased pats.	Dicumarol Controls	Acenocoumarine Deceased pats	Acenocoumarine Controls
Average	18.3	8.3	23.4	21.3	26	20
Median	17.5	18.0	22.0	19.0	25.0	20.0

Fig. 3 Number of thrombotests performed during the investigated year according to drug used.

groups were compared by means of the Student's t-test for paired observations.

This analysis showed that within each drug the variability in the two groups did not differ significantly However for dicumarol and acenocumarine the average variability was clearly wider than with phenprocoumon ($P < 0.001$).

The number of thrombotests is also a measure of the stability achieved: the more unstable the anticoagulation, the more thrombotests were done (Fig. 3). No important difference was found in the two groups.

In Table V the averages of the thrombotests and the median of the standard deviations are separately given for clinical recurrences and sudden deaths, according to age and in relation to the control group. Here, too no important difference was found.

A significant difference was found, however in the occurrence of non-lethal recurrences and of complications during the period investigated. These recurrences concern clinical infarctions as well as serious attacks of angina, recorded until the last three weeks before death (Table VI).

Table V *Average thrombotest values of the deceased patients, according to age and kind of death, and in relation to the averages of the living "partners"*

	Intensity		Stability	
	Deceased pats.	Controls	Deceased pats.	Controls
< 65 years	117.1	118.2	0.114	0.106
> 65 years	118.5	111.1	0.111	0.105
Sudden death	118.6	115.2	0.122	0.098
Death from clinical recurrence	117.0	114.2	0.103	0.114

DISCUSSION

The present investigation does not concern the evaluation of the usefulness of anticoagulants: both groups received anticoagulants, and a comparison with a placebo was not made. According to the results neither the intensity nor the variability of the anticoagulation differed significantly in the two groups. No evidence was obtained that patients with coronary heart disease died of a recurrence because anticoagulation had not been sufficiently intensive or stable. The deceased group included more patients suffering from cardiac failure, diabetes and recurrences before the fatal incident than the control group. Sudden death may be the result of electrical irritability developed during the original infarction (3). We also found no difference in intensity and stability between the patients who died suddenly and those who died a day or more after a recurrence. Nor was there any difference between patients younger and older than 65 years.

Table VI. *Complications and recurrences (non-fatal clinical myocardial infarctions and serious attacks of angina) in both groups during the period investigated*

	Deceased pats.	Controls
Diabetes mellitus	11	6
Heart failure	13	3
Recurrence	13	2
Total	37	11

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AUTOIMMUNE HEMOLYTIC ANEMIA IN ULCERATIVE COLITIS, CURED BY COLECTOMY

Report of a Case

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Abstract. A case of severe Coombs' positive hemolytic anemia in a 42-year-old woman with fulminant ulcerative colitis is described. Conservative treatment failed, total colectomy was performed on vital indication, and all symptoms disappeared. Coombs test also became negative.

Since the work by Lorber et al. (10) in 1955 14 patients with ulcerative colitis and anemia with antibody-coated red cells have been described, 13 of them female. Cases with antibody-coated red cells without anemia have also been published (4). The literature has been reviewed (3 7 9 11), and in some cases this type of anemia has improved after splenectomy However there seems to be only one briefly described case in which colectomy made the anemia and a positive direct Coombs test disappear (1). It thus should be of interest to describe a case of fulminant ulcerative colitis with a very severe hemolytic anaemia and a strongly positive direct Coombs test, completely cured by total colectomy performed on vital indication.

CASE REPORT

A woman, born in 1920, previously healthy apart from an acute pneumonia in 1959 and possibly a slight allergic rhinitis, developed an ulcerative colitis in 1961. Initially only proctitis, which improved on azulfidine. In August 1962 a severe relapse in connexion with respiratory infection. After about two weeks the patient had fulminant ulcerative colitis with frequent diarrhea and septic fever. She developed a severe anemia (Fig. 1) and, as the blood loss in the stools did not seem to be very large, a further investigation was performed and showed a hemolytic anemia with reticulocytosis and a positive direct Coombs test. Azulfidine and antibiotics had no effect on her condition. She was then transferred from her local hospital to the Department of Medicine, Karolinska sjukhuset X-ray showed ulcerations and dehaustration in the entire colon and corresponding typical findings on rectoscopy No splenomegaly Hb 5.0 g 100 ml.

Coombs test direct with the patient's erythrocytes was strongly positive ("titer" 1 4 096). Coombs indirect test was negative with standard red cells. Unfortunately no more detailed analysis of the antibodies could be performed. No Heinz bodies could be demonstrated in the red cells. Reticulocytes up to 18%, platelets 410 000–860 000, leucocytes about 12 000 with pronounced shift to the left. Total plasma bilirubin 0.63 mg 100 ml, 0.49 mg/100 ml of which unconjugated. Serum iron 0.158 mg/100 ml, total iron binding capacity 0.228 mg/100 ml. No LE cells were found. Serum GOT and serum GPT were normal. Serum protein 6.0 g 100 ml (2.2 g albumin/100 ml serum). Serum creatinine 0.9 mg/100 ml (Table I). Treatment with various antibiotics, betamethasone and ACTH (Fig. 1) caused only a slight improvement of her general condition but had little effect on the anemia or on the colon. In order to try to diminish the hemolysis and to break down macroglobulins, heparin and penicillamine were given without any lasting positive effect. In spite of the abnormal antibodies it was then considered necessary to give a series of blood transfusions. These did not cause any serious reactions and increased the hemoglobin. On the whole, however the situation remained as serious as initially and it was then decided to perform a total colectomy on vital indication.

The patient was transferred to the Department of Surgery Preoperatively and during the operation several blood transfusions were given. A total colectomy with ileostomy was performed without complications. The whole colon showed macro- and microscopically typical ulcerative colitis with ulcers, pseudopolyposis, edema and in some parts fibrosis (Fig. 2). No malignant changes were found. The small part of the ileum, which was resected, was normal.

Before the operation all medicines except betamethasone and ACTH had been withdrawn. Already one day after

Table I. *Distribution according to sex and age of 338 patients with acute myocardial infarction (bracketed figures: mortality rate)*

Age of patients (y)	Male	Female	Total
<50	28 (61)	6 (50)	34 (59)
50–59	55 (56 %)	9 (44)	64 (55%)
60–69	83 (58 %)	44 (57 %)	127 (58 %)
≥ 0	58 (55 %)	55 (55%)	113 (55)
Total	224 (57 %)	114 (54%)	338 (56%)

requested to supply information concerning all fatal cases of a.m.i. occurring outside hospital and requiring the co-operation and assistance of these institutions. Patients of this category and comprised in the present material, were in all cases autopsied.

6. Information was obtained from the National Health Service and the City Medical Officer concerning the total number of death certificates drawn up in November 1968 in which the cause of death was a.m.i.

7 Questionnaires were issued to all first-aid stations and ambulance drivers within the area concerned, in order to have record of the exact times at which ambulances were summoned and patients called for and handed over

If replies to the questionnaires were missing or incomplete, supplementary information was obtained by the author either by interviews or in the medical records.

MATERIAL

During the period under study diagnosis of true or suspected a.m.i. was established on 338 occasions, distributed over 224 male patients and 114 female. Of this number 190 (56%) died, viz. 128 male patients and 62 female. Accordingly the mortality rate among men was 57% among women 54%

The material may be divided into two main groups: (A) hospitalized patients (B) non-hospitalized patients.

A. This group comprised 183 patients (53%) in whom the diagnosis of a.m.i. was erified and who were still alive at the time of admission. All of these diagnoses were established on the basis of ECG, examination of enzymes and, occasionally on the basis of autopsy findings. The patients were referred either to coronary-care units or to combined intensive-care wards; some patients were admitted to general medical units. The mortality rate among hospitalized patients was less than 28%. Less than 50% of the patients were admitted to coronary-care units. The mortality rate among these patients was 22%. Among patients in combined intensive-care units the mortality rate was 29% and 39% among those in general medical units. The compatibility of these three groups of patients has not, however, been studied in further detail.

B. The group comprising non-hospitalized patients included a total of 1.5 patients (47%). Twenty-nine (9%) of these died in the ambulance taking them to hospital, 110 patients (33%) died at the locality where the attack occurred (i.e. locality other than hospitals, where the actual acute attack occurred and to which ambulance is called), and 16 patients (5%) were attended to and treated in their own homes by the family doctor: they remained at home throughout the period of illness. Among these 155 patients verification of the diagnoses as possible only in 56 cases, iz. in 24 who died in the ambulance taking them to hospital, in 19 who died at the scene of the accident immediately before being taken to hospital, and fi who wer dead at the time when found. In all of the latter cases the diagnosis of a.m.i. was verified at autopsy In further eight patients who ere treated in their own homes by the family doctor the diagnosis was verified on the basis of ECG and examination of enzymes. As regards the remaining 99 patients in this group, diagnosis of a.m.i. could not be verified and was established exclusively on the basis of a very typical past medical history and characteristic course of the disease. Among these 99 patients there were 86 who died at the locality other than hospitals, where the attack occurred, and five who died in the ambulance taking them to hospital. None of these patients were autopsied. In addition, eight patients are included who were treated in their ow homes by the family doctor: supplementary examinations by which the diagnosis might otherwise have been erified were not performed.

Table I records the distribution according to sex and age of the patients with a.m.i., illustrating at the same time the rate of mortality in the arious age groups (bracketed figures) The male to female ratio as 1.96

For all patients in this series it was possible to determine the time interval bet een onset of primary symptoms of a.m.i., the exact time at hich the medical officer or the ambulance was called for the time of arrival

Table II. *Relation between onset of primary symptoms of acute myocardial infarction and occurrence of death according to scene of accident 190 fatal cases of acute myocardial infarction*

Time interval between first symptom and death	Death at scene of accident	Death in ambulance	Death upon admission	Total
<15 min	46	8	0	54
15–30 min	32	9	0	41
30–60 min	30	4	3	37
1 2 h	2	4	4	10
2–4 h	0	4	4	8
4–12 h	0	0	5	5
12 24 h	0	0	6	6
24–48 h	0	0	5	5
3–4 d	0	0	6	6
5–8 d	0	0	7	7
>8 d	0	0	11	11
Total	110 (58%)	29 (15 %)	51 (27 %)	190

of the latter at th scene of the accident, and the time hen the patient was admitted or death occurred.

If ambulances ere called for on an emergency basis and medical officer had not had an opportunity to see the patient in advance, the time interval between onset of symptoms and admission to hospital ould only be about 2 h. Generally these patients had been taken acutely and severely ill, which is apparent also from the fact that 55 (79%) out of the 70 in the group died. Death at the scene of the accident occurred on 19 occasions, 20 died in the ambulance taking them to hospital, and 16 died upon admission. The time interval between onset of the primary symptoms of a.m.i. and the calling for ambulances was rather brief also in this group, on an average 90 min, the interval between the calling for and arrival of ambulances was equally brief, averaging some 5 to 10 min.

As regards the 150 patients who were hospitalized by medical officer or whom he applied to have hospitalized, the time interval between onset of symptoms and summoning of the doctor averaged 8 h. Patients of this category ere rarely taken to hospital in an emergency ambulance as opposed to patients of the former category for which reason the time spent on waiting for the ambulance and driving to the hospital as prolonged, on an average by 2 h. Death in the ambulance taking these patients to hospital occurred only on nine occasions; 32 died on admission to hospital. Table II records the time interval between onset of the primary symptoms of m.i. and the occurrence of death of all of the 190 patients in the series; also the location at hich death occurred. In the vast majority of cases the patients died at home, and death had usually occurred before an ambulance or medical officer had been called for. It also appears from Table II that death due to m.i. at locations other than hospitals occurred in 73% of the total number of fatal cases, 15 f all the dead patients died in the ambulance taking them to hospital. I will finally be seen from the table that 70% of the patients died within 1 h after onset of the primary symptoms, hile 83% died within 12 h after onset.

The past medical histories of 231 patients ere very detailed. Of this number 183 were still alive at the time of admission and 48 died before arrival in hospital. Data concerning these patients are recorded in the following three tables.

Table III shows the time of onset of the primary symptoms of a.m.i. The distribution as rather uniform all round the clock and, according to the investigation, there was no significant accumulation of cases of m.i.

Table III *Time of onset of the primary symptoms of acute myocardial infarction in 231 patients*

Time of onset (h)	No. of pats. (%)
00–08	75 (32%)
08–16	71 (31 ,,)
16–24	85 (37)
Total	231 (100 ,,)

Table IV *Scene of accident here the myocardial infarction occurred*

Scene of accident	No. of pats. (%)
At home	197 (84%)
At work	17 (7 ,,)
In the street, etc.	17 (7 ,,)
Total	231 (100)

Table V *Classification of final diagnoses of 348 patients who were alive at the time of admission on a suspicion f acute myocardial infarction*

Final diagnosis	No. of pats. ( )
Acute myocardial infarction	183 (52 ,,)
Cardiac lesions other than acute myocardial infarction	117 (34)
Extracardiac diseases	48 (14)
Total	348 (100)

on special days of the eek or periods ithin the month. Out of the 231 patients, 66 (29) had experienced one or more episodes of m.i., hich in all cases had been verified during hospitalization, and 129 (56%) had been suffering from angina pectoris for at least one month prior to the present episode. A previous occurrence of m did not significantly levate the mortality rate. I 139 patients (60%) the acute disease had been preceded by heterogeneous types of prodromes such as non-characteristic precordial oppression, intensified sensations of fatigue, or general malaise persisting from 24 to 48 h. The mortality rate among these patients as 47%, as opposed to the 56 otherwise found in the entire series.

Table IV illustrates the whereabouts of patients at the time of occurrence of the acute episode. The patients ow homes are seen to predominate distinctly.

In addition to the 183 patients with erified diagnoses of a.m.i. ho ere still alive at the time f admission, further 165 patients ere hospitalized during the month, these patients ere admitted on suspicion of m.i., but the diagnoses could not be verified. The final diagnoses of the 348 patients ho ere still aliv at the time of admission are recorded in Table V. In the absence of a.m.i., the most common symptoms were other cardiac affections of more or less severe character.

A total of 190 patients ith a.m.i. died in the course of Nov. 1968. Autopsies were performed on 92 (48%) of them, i.e. on 96% of the patients who died in hospital, on 82% of those ho died in the ambulance taking them to hospital, and on 17% f the patients ho died at localities other than hospitals immediately upon occurrence of the attack. The sites of the myocardial infarctions, determined on the basis of autopsy findings, com-

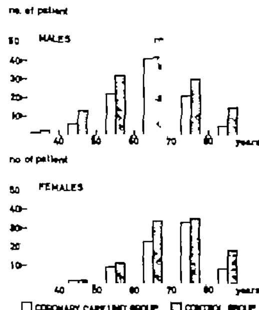


Fig 1 Distribution of patients according to sex and age.

included in this study on the basis of exactly the same criteria, namely·

1. Admission to the hospital under suspicion of acute myocardial infarction of less than seven days duration.
2. Typical history of acute onset of chest pain and/or syncope and/or acute pulmonary oedema.
3. Typical ECG changes in at least two standard leads and one precordial lead.
4. Characteristic changes in the ECG during the stay in hospital.
5. Transient elevation of S-GOT and α_1-fraction of LDH in the serum.
6. In the event of death without the above criteria having been fulfilled, the diagnosis had to be confirmed by autopsy

Patients with cardiac arrest prior to admission were not included.

The diagnostic assessment in each individual case was in the hands of the three authors. Thus the diagnoses was not based upon the estimate by the various departments.

All patients remained in hospital for at least 28 days after the onset of symptoms. The patients in the coronary-care unit were mobilized in bed from the first day and transferred into an arm-chair as soon as they had been relieved of pain and their blood pressure had been stabilized. In the departments of general medicine the patients were allowed to get up around the 14th day. Anticoagulant therapy and oxygen were not administered routinely

In the coronary-care unit patients with proved myocardial infarction were monitored continuously for minimum of seven days after the onset of symptoms. The observation and treatment of the patients was in the hands of specially trained staff acting in accordance with a specific set of therapeutic principles and procedures. ECGs were transmitted to continuously running 8-channel tape recorder and analysed daily for arrhythmias on an oscilloscope.

After the monitored period the patients remained in the unit for another three days before being transferred to one of the three departments of general medicine.

The treatment of cardiac arrest has for several years been performed on the same principles in all departments of the hospital. Every physician and every nurse who joins the hospital staff receives instruction in resuscitation. As all modern medical and prevalent technical aids are available in all the medical departments, the patients received—at least theoretically—the same kind of treatment in the departments of general medicine as in the coronary-care unit.

In this way we have been able to obtain a material of patients with acute myocardial infarction, selected arbitrarily without limitation of any kind, and treated simultaneously in the same hospital on the same therapeutic principles. This material was divided at random into two groups, one of which received "normal care and the other continuous observation and treatment by specially trained staff, as rule the nurses who were the only personnel constantly present in the unit. In most cases, therefore, it was the nurse who detected the various kinds of arrhythmias and who had to treat them, at least in the event of sudden onset.

During the period of investigation 328 patients ere admitted to the coronary-care unit and 484 to the two departments of general medicine because of suspicion of myocardial infarction.

According to the established criteria, the diagnosis was confirmed in 171 (52.2%) of the cases in the coronary-care unit (the *coronary-care unit group*) and in 244 (50.4%) of the cases in the departments of general medicine (the *control group*). Of the patients in the coronary-care unit 96 were males (56.1%) and 75 females (43.9%), while the control group comprised 144 males (59.0%) and 100 females (41.0%). The male/female ratio was 1.3 and 1.4 respectively

No patient with coronary occlusion confirmed *post mortem* had previously been excluded from the material on the basis of the clinical criteria. Thus, in no case was there question of negative clinical and positive autopsy findings.

Sex and age distribution in the two groups is given in Fig. 1 The age distribution was the same the average age of the males admitted to the coronary-care unit being 64.2 as compared with 64.7 in the control group. Among the females the average age was 70.0 in both groups.

In respect to *important co-existing diseases*—e.g. arterial hypertension, diabetes mellitus, etc.—the two groups showed no significant differences apart from preponderance of obesity in the coronary-care unit group.

A *history of coronary occlusion* was elicited in 32 patients (18.7%) of the coronary-care unit group and in 67 (27.5%) of the control group. This difference is hardly significant.

Duration of present illness before admission is compared in Table I. In 41.5% of the patients admitted to the coronary-care unit the duration was less than three

hours at the time of admission, as compared with 25.4% of the control group. This difference is significant, $0.01 > p > 0.001$.

Clinical condition on admission. The group named acute pulmonary oedema comprises only patients who exhibited, apart from severe pulmonary congestion, typical foam at the mouth. From Table II it is apparent that relatively larger number of patients with cardiac decompensation were admitted to the coronary-care unit than to the control departments. This difference is statistically significant, $p < 0.001$. No differences were found between the other parameters.

Localization of infarctions according to the ECGs is given in Table III. In the coronary-care unit group the site is listed as indefinite in 14%. This is taken to mean patients with bundle branch block or with arrhythmias of nature which made it impossible to locate the infarction.

To render the comparison more reliable, the sites were classified only into anterior, posterior, and indefinite. The fact is that in the majority of cases in the control group only rather few ECGs had been obtained, and as routine only in three standard and two precordial leads, viz. C_1 and C_4. This inaccuracy is reflected in the relatively large number of indefinite ECGs. The table clearly shows significantly larger number of posterior infarctions in the control group than in the coronary-care unit group.

Arrhythmias considered to require treatment occurred in 67% of the 171 patients in the coronary-care unit. In the control group, in which continuous recording was not practicable, arrhythmias were diagnosed on the basis of the ECG tracings and cardiac auscultations in about 30% and antiarrhythmic treatment was instituted in 17%.

RESULTS

For the patients admitted primarily to the coronary-care unit, the 28 days observation period included also, as mentioned above, the subsequent stay in the general wards. To assess the value of observation and treatment in the coronary-care

Table I. *Duration of symptoms before admission*

Duration of symptoms	Coronary care unit group (171 pats.) % of pats.	Control group (244 pats.) % of pats.
< 1 hour	16.9	9.0
< 3 hours	41.5	25.4
< 6 hours	55.8	41.0
< 12 hours	68.5	57.2
< 24 hours	77.3	66.3
< 36 hours	83.1	72.5
< 48 hours	86.0	75.4
< 72 hours	90.7	82.8
< 7 days	100.0	100.0

Table II. *Clinical condition on admission*

Clinical condition	Coronary care unit group (171 pats.) % of pats.	Control group (244 pats.) of pats.
Congestive heart failure	52.6	38.1
Pulmonary oedema	4.7	6.6
Severe angina	49.1	43.4
Shock (clinical)	21.6	21.3
Cardiac arrest (shortly after admission)	1.8	1.2

Table III. *Localization of myocardial infarctions as determined by ECG*

Localization	Coronary-care unit group No. of pats.		Control group No. of pats.	
Anterior wall	81	47	78	32
Posterior wall	66	39	115	47
Indefinite	24	14	51	21
Total	171	100	44	100

unit and in the control departments, the mortality in the 2 groups within this period was calculated.

During the observation period 30 of the 171 patients admitted with acute myocardial infarction to the coronary-care unit (or 17.6%) died as compared with 101 of the 244 patients (41.4%) in the control group. The difference in the total mortality is highly significant, $p < 0.001$.

The variation of the mortality with sex and age is shown in Table IV. For women admitted primarily to the coronary-care unit the overall mortality was 25.3% that in the control departments 50.0%. For males the corresponding rates were 11.5% and 35.4%. For patients under 55 years of age the mortality rate was 0 in the coronary care unit and 30.6% in the control departments. For patients under 70 years of age the rates were 13.5% and 35.6%. Independently of sex and age, there was a significant reduction in mortality in the coronary-care unit group as compared with the control group. For women over 70 the mortality was 29.3% and 58.5% respectively as compared with 15.4% and 40.0% for men in the same age range. Owing to the striking mortality among, and the considerable proportion of, elderly women in both materials, 40% of all

Table IV *Distribution of patients and deaths according to sex and age*

	Males						Females					
	Coronary care unit group			Control group			Coronary care unit group			Control group		
Years of age	No. of pats.	No of deaths		No of pats.	No. of deaths	%	No. of pats.	No. of deaths	-	No. of pats.	No. of deaths	%
<40	1	0	0	2	0	0	0	0	0	0	0	0
40–49	6	0	0	13	2	15	2	0	0	2	2	100
50–59	22	2	9	32	12	38	9	3	33	11	4	36
60–69	41	5	12	52	19	37	23	4	17	34	13	38
70–79	21	2	10	30	11	37	33	8	24	35	19	54
>80	5	2	40	15	7	47	8	4	50	18	12	67
Total	96	11	11	144	51	35	75	19	25	100	50	50

deaths in the coronary-care unit group and 31% of all deaths in the control group occurred among women over 70 years of age. The corresponding values for males were 13% and 17% respectively

Table V shows the correlation of the mortality to the duration of illness prior to admission. By far the greater part of the patients in both groups were admitted within 24 hours of the onset of the first symptoms. The mortality for these patients was 17% in the coronary-care unit and 36% in the control departments—corresponding largely to the overall mortality in the groups.

A history of angina pectoris did not influence the interval between the onset of symptoms and the time of admission.

As already mentioned, fewer patients with a history of myocardial infarctions were admitted to the coronary-care unit than to the control departments. However it may be seen from Table VI that the difference between the two groups is manifest solely in the *number of patients with a* history of coronary occlusion more than two years prior to the present infarction. Myocardial infarction less than two years before the present one was in fact equally common in both groups and entailed an increased mortality ($p < 0.05$). However the difference between the two groups still remains significant.

Table VII gives the mortality among patients admitted with cardiac failure, acute pulmonary oedema, or shock. It will be noted that cardiac failure per se did not increase the mortality among patients admitted to the coronary-care unit. Patients with acute pulmonary oedema and shock showed a considerably increased mortality in both groups, but this increase was less marked in the group admitted primarily to the coronary-care unit. The stated values for shocked patients refer to the condition prior to administration of analgesics. After the patients had been relieved of pain,

Table V *Mortality in relation to duration of symptoms before admission*

Duration of symptoms before admission	Coronary care unit group			Control group		
	No. of pats	No. of deaths		No. of pats.	No. of deaths	%
<1 hour	29	8		22	17	
1–3 hours	43	7		40	16	
3–6 hours	23	2		43	7	
6–12 hours	23	4		25	11	
12 24 hours	15	2		32	8	
Total <24 hours	113	23	17	162	59	36
1 3 days	42	6	14	41	20	49
4–7 days	16	1	6	41	22	54
T tal <7 days	171	30	17.6	244	101	41 4

Table VI. *Interval between present and previous myocardial infarction in relation to mortality rate*

Interval from previous myocardial infarction (months)	Coronary care unit group			Control group		
		Deaths			Deaths	
	No. of pats.	No.	%	No. of pats.	No.	
<1	2	0	0	3	3	100.0
1 4	23	6	26.1	37	21	56.8
>24	7	1	14.3	28	12	42.9
Total	32	7	21.9	68	36	52.9

20 patients (12%) in the coronary-care unit still exhibited clinical shock with a systolic blood pressure below 100 mmHg. Eleven of these patients (55%) died, seven of them in cardiogenic shock. Such a distinction was not possible i the control group, as the case records did not state when analgesics were administered in relation to the determinations of the blood pressure.

Correlation between the site of the infarction and the mortality was assessed on the basis of the ECG tracings (Table VIII). Among patients admitted to the coronary-care unit the mortality was highest in those with uncharacteristic ECG findings and lowest in those whose EGGs indicated posterior-wall infarction. Among the patients admitted to the control departments the mortality was highest in the group showing ECG signs of posterior wall infarctions. Since, as already mentioned, there was a preponderance of this category in the control group, we investigated how this factor affected the overall mortality in the two groups. Correction for skewness in the occurrence of ECG changes entailed a reduction of the mortality to 14.5% and 38.5% respectively Thus the difference between the mortality rates still remained unchanged.

Each of the two compared groups included three cases of pulmonary infarction. In addition, three patients in the coronary-care unit, but only one of the control group, died of pulmonary embolism.

Among the patients admitted to the coronary care unit there were three cases of oliguria-anuria. Two of these patients succumbed. In the control group there was one case of oliguria-anuria, and the patient survived. In the coronary-care unit there was one case of cerebral damage, which occurred also in eight patients of the control group. All these patients died.

Re-infarction occurred during the stay in hospital in two patients of the coronary-care unit group and in six of the control group.

The lower mortality rate in the coronary-care unit group could not be ascribed to more effective treatment of cardiac arrest, since among 11 patients treated by external cardiac massage or DC defibrillation in the coronary-care unit 8 died, as compared with 10 out of 12 patients in the control group

Table VII. *Relation between clinical condition on admission and mortality rate*

Clinical condition	Coronary care unit group (171 pats.)		Control group (244 pats.)	
	Patients ( )	Dead (%)	Patients ( )	Dead ( )
Congestive heart failure	53	18	38	50
Pulmonary oedema	5	37	7	81
Shock	22	41	21	75
(Shock after administration of analgesics)	(12)	(55)	—	—

Table VIII *Localization of myocardial infarctions by ECG in relation to mortality rate*

Localization	Coronary care unit group (171 pats.)		Control group (244 pats.)	
	Cases ( )	Dead ( )	Cases ( )	Dead (%)
Anterior wall	47	19	32	27
Posterior wall	39	16	47	51
Indefinite	14	25	21	41
Total	100	—	100	—

DISCUSSION

Whether the demonstrated differences in mortality constitute an applicable measure of differences in observation and treatment in the coronary-care unit and in the control departments depends upon the comparability of the two groups of patients in respect to parameters other than the departments in which they were treated.

The present study comprises all patients admitted with acute myocardial infarction, irrespective of sex, age, severity of symptoms, or complicating diseases, and an attempt was made to obtain a completely random distribution in the two groups. Indeed, the sex ratio and age distribution were the same in both groups and in respect to other parameters, too, a satisfactory agreement was found. However certain differences were also present, presumably due to the limited number of patients included in the study For instance the coronary-care unit received a relatively larger number of patients with obesity a shorter duration of symptoms, and cardiac failure, as well as a larger number with anterior infarctions than did the control departments. These differences, however were not so marked that they might be expected to cause essential differences in the therapeutic effect. Since, moreover they invariably related to the group treated in the coronary-care unit, they can at least not have contributed to the better results obtained in it. In the coronary-care unit the mortality was higher among patients with anterior/antero-septal infarctions than among patients with posterior-wall infarctions. This distribution of the mortality corresponds to the findings in a much larger series (8)

All considered, the study shows that among patients admitted to the coronary-care unit the mortality was less than half that found in the control departments. The difference between the absolute values for mortality was about 24%. This gain of 24 survivors in 100 admitted patients appears to be constant also for the sub-groups of sex and age. Accordingly there is no justification for limiting admission to a coronary-care unit on sex or age criteria.

It is probably not relevant to compare the present results in an unselected material of patients having acute myocardial infarction with those of previous studies in which the composition and distribution of the material has been different. However our results agree with those recently obtained in a similar controlled study from Stockholm (7).

The mortality rate of the control group is of the same order of magnitude as found by other investigators in the Scandinavian countries (4 17) and as previously reported from this hospital (6). Further Mosbeck and Dreyer (13) found a mortality rate of 41% for all Denmark in 1965 and Haghfelt (5) a mortality rate of 39% in the city of Copenhagen in 1968 among patients with acute myocardial infarction treated in general medical departments. These mortality rates seem to be somewhat higher than reported from the UK and USA (11 14 16), which presumably is due to the fact that almost all cases of myocardial infarction in the Scandinavian countries are treated in hospital and that the patients are admitted to hospital at a very early stage of the disease.

The longer duration of illness in patients admitted to the control group might partly be due to the procedures of admission. The patients of the coronary-care unit were transported directly to the unit, whereas the patients of the control group were taken primarily via the central admission department, which delayed the admission to the general ward departments by 15 min on average. This discrepancy is, after all, to the advantage of the control group, as eventual cases of death occurring before arrival in the departments had been excluded.

The better prognosis for patients in the coronary-care unit is not due to the ideal possibilities of immediate treatment of cardiac arrest, as the mortality among these patients was around 70% largely the same as in the control group

In our opinion there are other reasons for the better results in the coronary-care unit. The most important one is presumably the close observation of the patient's clinical condition and the early institution of treatment in the event of disturbances in rhythm and/or circulatory failure at a stage when such treatment has a possibility of being successful.

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FOUR CASES OF MASSIVE DIGITALIS POISONING

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Abstract. Four cases of massive digitalis poisoning are described. One of the patients died. Potentially fatal arrhythmias were seen in all four cases. Failing response to conventional drug therapy was observed. Pacemaker treatment was applied in three cases and defibrillation in the fourth. Three of the four cases were thus successfully treated. The value of handling these patients in a unit capable of providing intensive cardiac care is underlined. The role of hyperkalemia in digitalis poisoning, its prognostic significance and position in the therapeutic discussion is ventilated.

Therapy in massive digitalis poisoning includes atropine, potassium supplement, EDTA as well as antiarrhythmic drugs like ajmaline, diphenylhydantoin, lignocaine, procaine amide, beta-blocking agents and cardiac pacing (1, 4, 16, 18, 21, 23, 24, 25). Several reports on massive digitalis poisoning have appeared in the literature either as case reports (2, 4, 5, 6, 17, 22, 23) or as accounts of series and reviews on the topic (3, 7, 10, 15, 19).

Recently we have seen four episodes of massive digitalis poisoning in three patients (Table I). They were continuously monitored in a coronary care unit. In all four cases ventricular standstill or ventricular fibrillation occurred. Three of the four episodes were successfully treated with pacing or defibrillation. The cases are presented and discussed.

CASE REPORTS

Case 1

Woman aged 60. Sporadically on peroral diuretics and digitalis because of moderate heart failure. No other medication. She took 10 mg digoxin with suicidal purpose and was admitted to the hospital with nausea and vomiting 2 hours later. Gastric lavage was performed.

The patient was transferred to the coronary care unit. She was delirious and showed generalized jerking movements. The heart rate was 100/min and regular. There were no rales. BP 120/70. An ECG showed sinus rhythm and slight ST-T depression in the left precordial leads. Serum potassium 5.5 mEq/l. Monitoring over the next hours showed periods of supraventricular tachycardia with second degree A-V block. At times nodal rhythm was observed. Three hours after admission the systolic blood pressure had fallen to 85 and the patient was oliguric. The treatment was oxygen and 5.5% glucose drip with potassium supplements. Attempts to reduce hypocalcemia with NaH_2PO_4 orally were stopped because of vomiting. Seven hours after admission there were occasional supraventricular tachycardias with ventricular rates of 140–150/min. After 2 mg of propranolol intravenously long periods of slow ventricular activity 30–40/min, followed. A bipolar electrode was therefore introduced transvenously into the right ventricle and on demand pacing at 80 beats/min was started. The threshold for myocardial stimulation was 1.8 V. Peritoneal dialysis was started 12 hours after admission with potassium 4 mEq/l added to the dialysing fluid. Seventeen hours after admission the patient suddenly developed ventricular fibrillation. After a few seconds this was followed by asystole, and there was no response to pacemaker stimuli of maximum voltage. Transthoracic pacing proved equally unsuccessful.

Post mortem examination showed both electrodes to be in adequate position in the right ventricle. There were diffuse petechial haemorrhages in the myo- and endocardium of the left ventricle. The coronary arteries showed slight atherosclerotic changes but there were no signs of myocardial infarction.

Case 2

Man aged 71 treated at our hospital for a cerebrovascular accident and atrial fibrillation. He was readmitted five days after discharge, complaining of nausea and vomiting. He had probably taken 24 mg digoxin and 5 g slow release quinidine during five days.

On admission to the coronary care unit he was uncooperative, incoherent and aggressive. BP 115/70. ECG showed atrial flutter with varying degrees of A-V block II, ventricular rates 20–90/min (Fig. 1). During the periods of bradycardia there was no response to atropine intravenously in a total dose of 1.5 mg. Thirteen hours after admission ventricular tachycardia suddenly intervened, ending in ventricular fibrillation. This was successfully defibrillated (400 joules DC) and lignocaine infusion

Table I. *Clinical data for the four reported cases*

Case	Sex	Age (years)	Digoxin intake	Basic rhythm	Arrhythmias	Max. serum potassium (mEq/l)	Electrical therapy	Outcome
1	♀	60	10 mg	SR	SVT NR, AV II, VF ASY	5.5	Pacing	Died
2	♂	71	24 mg in 5 days	AFL	VT VF	7.8	DC-shock	Survived
3	♂	71	12.5 mg	AFI	AFL, NR, ASY	6.0	Pacing	Survived
4	♀	16	15 mg	SR	AE, AV II	4.7	Pacing	Survived

SR, sinus rhythm. AE, atrial ectopics. AFL, atrial flutter. AFI, atrial fibrillation.
SVT, supraventricular tachycardia. NR, nodal rhythm. AV II, second degree A-V block.
VT, ventricular tachycardia. VF, ventricular fibrillation. ASY, asystole.

was started. During an uneventful course the ventricular rate gradually increased over the next days and the patient was discharged nine days after admission.

Case 3

Same patient as above (case 2) was readmitted six weeks later because of acute arterial insufficiency in the left leg. In the ward the patient on one occasion took his own digoxin tablets in an estimated amount of 12.5 mg.

His mental state deteriorated and he started vomiting. The pulse slowed and he was transferred to the coronary care unit. He was again incoherent and aggressive. BP 120/80. There were no clinical signs of heart failure. The

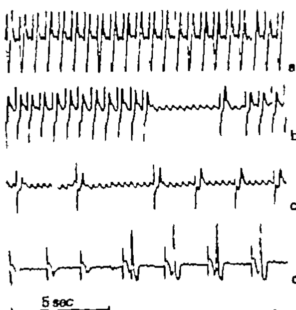


Fig. 1. Atrial flutter with 2:1 block (*a*), and short periods of marked blocking, occurring abruptly (*b*). Later with progressive blocking the heart rate slowed (*c*), and occasional, in all probability ventricular, ectopic beats (*d*), always coming closely after supraventricular beat.

ECG showed *atrial fibrillation and at times nodal bradycardia*. Extremely low ventricular rates developed, 13–30/min, 30–40 hours following ingestion. There was poor *response to methyl scopolamine (total 1.0 mg) intravenously*. Serum potassium was 6.0 mEq/l and no potassium supplement was given. Forty-five hours after the digoxin ingestion several periods of excessive bradycardia intervened, associated with Stokes-Adams attacks. The longest ventricular standstill registered was 16 sec (Fig. 2). Scopolamine and isoprenaline gave only transient relief, and further Stokes-Adams attacks occurred, responding to blows on the chest. No spontaneous idio-ventricular beats terminated the standstills. A bipolar electrode was introduced transvenously into the right ventricle and pacing *on demand was started at 80 beats/min. The threshold for* myocardial stimulation was 1.4 V. Pacing could be discontinued after 2 days and the patient was discharged 7 days later with atrial fibrillation and normal ventricular rates.

Case 4

A 16-year-old previously healthy girl. After quarrel with her boyfriend she slashed her wrists and took 8 sleeping pills and about 15 mg digoxin belonging to her father. On admission to the coronary care unit she complained of nausea and vomited. She was orientated but depressed. Physical examination was normal. BP 120/70. An ECG showed sinus rhythm, 55/min, with moderate ST depression and biphasic T-waves in the left precordial leads. Serum potassium 4.1–4.7 mEq/l. She was given potassium supplements intravenously 80 mEq daily. The patient remained in irregular supraventricular rhythm regarded as sinus rhythm with occasional atrial ectopic activity until she suddenly 32 hours after ingestion, developed AV-block II followed by three episodes of ventricular standstill of 4–7 sec duration (Fig. 3). A bipolar electrode was introduced transvenously into the right ventricle. The threshold for myocardial stimulation was 0.6 V. Pacing was continued on demand at 80 beats/min for 24 hours. Hereafter normal sinus rhythm. ECG four days after arrival showed marked T-wave inversions in the left precordial leads.

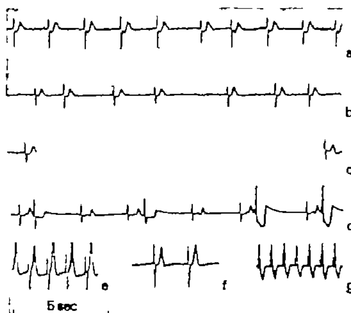


Fig. 2. Initially atrial fibrillation with slow heart rate (*a*), showing further lowing (*b*), and occasionally extremely long PR-intervals, as (*c*) 15.6 sec. No escape beats seen. A few ventricular ectopic beats at times (*d*). Isoprenaline infusion increased the heart rate and regularised the rhythm (*e*), but the effect soon vanished; *f* registered one minute after infusion. *g*, pacemaker-induced heart rhythm, rate 90/min.

DISCUSSION

Four episodes of massive digitalis poisoning have been described. The approach to the management of these patients has followed the general outlines. We made use of monitoring, pacing and defibrillation. Antiarrhythmic and vagolytic drugs were of minor value in these patients, all of whom had ingested very large doses of digitalis.

Beta-blocking agents have been advocated in the treatment of moderate digitalis poisoning (24, 25). A small dose of propranolol resulted in a pronounced bradycardia in one of our cases (no. 1), which is in accordance with an observation made earlier (26) of undue sensitivity to propranolol in digitalis poisoning.

It is our impression that the monitoring and other specific facilities available in a coronary care unit have been of great benefit. By these means potentially lethal arrhythmias could be discovered immediately and treated efficiently.

In three cases, pacemaker treatment was undertaken. As ventricular standstill occurred in three out of four cases, it might be suggested that a pacemaker electrode should be introduced prophylactically immediately after admission.

One patient died in the presented series. A mortality of 14 out of 70 has previously been reported (14). According to that report, a high age of the patient, the presence of cardiac disease, hyperkalemia, renal or hepatic insufficiency and "signs of myocardial irritability" are associated with a poor prognosis.

At autopsy the heart of the patient in case 1 presented a picture with scattered hemorrhages. This phenomenon has been described by Dearing et al. (9) in cats. In man, a similar picture has

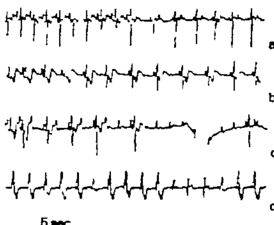


Fig. 3. Irregular supraventricular rhythm, considered as sinus rhythm with occasional atrial ectopic activity (*a* and *b*). Suddenly atrioventricular block degree II interfered, followed by repeated ventricular standstills of 4–7 sec duration (*c*). After pacemaker application (*d*) interference was seen at a heart rate of 72 beats/min.

been described in one case (19), but has been absent in others (3).

On reviewing continuously recorded ECGs for cases 2, 3 and 4 strikingly few ventricular ectopic beats were observed, and always closely after a preceding supraventricular beat. Even in extreme bradycardia, surprisingly few idioventricular escape beats occurred. Moderate doses of digitalis have been shown to have a negative effect on ventricular automaticity (8 12, 13). Toxic doses, however seem to have the opposite effect (20 27).

Of special interest is the hyperkalemia noted in our cases (cases 1 2, 3). High potassium values after digitalis poisoning have been reported elsewhere (3 10 14 17 23). Apart from the hyperkalemia, no other specific electrolyte disturbance nor acid-base disequilibrium has been observed clinically (14) Observations have been made that toxic doses of digitalis may decrease the amount of potassium in the heart muscle fibre and probably also in other muscle fibres (26). It is suggested that a mechanism for this could be the digitalis-mediated inhibition of cell membrane ATP-ase that supplies energy for effective transport of ions across the cell membrane (20).

Hyperkalemia might also be due partly to oliguria following hypotension. This complication has been reported in a rather high frequency (14) Serious degrees of hyperkalemia can be treated by dialysis, which, however is useless in eliminating the digitalis glycoside (11), as is also indicated by our first patient. Digitalis intoxication could theoretically be counteracted by decreasing extracellular calcium. Treatment with this purpose has been reported in one case with an encouraging result (16) Perorally administered NaH_2PO_4 in an attempt to induce hypocalcemia in the deceased patient (case 1) was discontinued because of vomiting.

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TREATMENT OF ANGINA PECTORIS WITH BETA RECEPTOR BLOCKING AGENTS

Effects of Long-term Treatment and Treatment with Sustained Release Tablets

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Abstract. Nine patients with angina pectoris had been treated between two and three years with the beta blocking agent alprenolol when they agreed to withdrawal of treatment and introduction of double-blind cross-over trial with placebo. *All had more attacks on placebo than on alprenolol.* Six patients had no attacks on alprenolol, but angina pectoris returned when they were on placebo. The same nine patients, one year later, compared the ordinary tablet of alprenolol (100 mg 4) and sustained release tablets (200 mg 2). No significant difference was found in frequency of attacks of angina pectoris or in subjective ranking of these two drugs. *It is concluded* that β-receptor blockade as treatment of angina pectoris cannot be withdrawn even after long period of treatment. Furthermore, the sustained release tablets were effective against angina pectoris, because they showed no difference from alprenolol in ordinary tablet form, and the latter were much more effective than placebo in the same patients.

Treatment of angina pectoris with β-receptor blocking agents has been found effective in several well-controlled trials (3). The effect is believed to be caused by a decrease in myocardial oxygen consumption due to a decrease in arterial blood pressure, heart rate and contractility and with some of these agents a decrease in cardiac output (3, 4). Myocardial analgesia, due to the local anesthetic effect of the drugs, is probably not responsible (2).

Improvement of angina pectoris allows an increased physical activity. Increase of everyday physical activities such as walking etc. might perhaps result in physical training of the patient. Physical training of patients with angina pectoris causes improvement because of a lowering of pulse rate, blood pressure and cardiac output on submaximal work loads (5, 6, 8). Theoretically therefore after long-term treatment, β-receptor blocking agents might be thought to be unnecessary because the effects seen on the patients might be a consequence of physical training made possible by the drug treatment. In order to test this possibility the effects of a β-receptor blocking agent (alprenolol, Aptin® Hässle, Goteborg, Sweden) were tested after several years of treatment in a group of patients with angina pectoris. On the same patients the effect of a sustained release tablet with the same amount of the drug mentioned was tested in order to see whether administration of the tablets twice a day could replace administration four times daily (7).

MATERIAL AND METHODS

Nine patients took part in both trials. These patients had participated in previous trials (1, 2) and taken alprenolol for 24 to 38 months in dose of 100 mg four times daily. They had been controlled in ordinary out-patient routine about every sixth week during this period. Before the present trials they were told that it was possible that the drug was no longer necessary and that, during period, blind tablets or the drug would be given. In the trial with the sustained release tablets the patients also agreed to testing tablet which, if effective, had considerable practical advantages.

The patients taking part in the trials all had typical angina pectoris coming on with physical exercise, being relieved immediately after rest or after nitroglycerin. All had suffered myocardial infarction between 1 and 5 years before the trial. They were all men between the ages of 45 and 73 years. One patient suffered from essential hypercholesterolemia (case 2) and two from essential hypertension treated with saluretics (cases 6 and 8).

The trial method has been described in detail previously (1, 2). In short, patients were seen weekly or every second week during identical procedures, including taking history and making physical examination. Blood samples

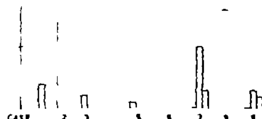


Fig. 1. Number of attacks of angina pectoris per two weeks in individual patients in a double-blind cross-over trial of alprenolol (100 mg 4 times daily) and placebo. Patients treated with alprenolol 4–33 months before this trial. White columns, placebo; black columns, alprenolol, 100 mg 4 times daily.

were taken on the last visit in each test period and determinations were made of bilirubin, alkaline phosphatase, thymol turbidity, GOT, GPT, hemoglobin, white blood cell and blood platelet counts and plasma creatinine.

The first trial was performed during February through April, 1969, and the second during March through April, 1970. The first trial consisted of two 3-week periods during which either alprenolol (100 mg four times daily) or placebo tablets of identical taste and appearance were given. The patients were instructed to swallow the tablets immediately with a glass of water in order to avoid bias because of anesthetic effects of alprenolol on the mucous membranes of the mouth. In order to avoid possible carry-over effects, the two last weeks of the trial periods were evaluated. Drug and placebo were given by double-blind cross-over technique.

The second trial was identical to the first except that two sorts of tablets had to be given in each period. These tablets were either a combination of alprenolol (100 mg) and placebo simulating the sustained release tablets or sustained release tablets of alprenolol (Aptin Duretter® 200 mg) and placebo simulating alprenolol tablets. Alprenolol or its placebo was taken four times daily (7 a.m., noon, 5 and 10 p.m.) and the sustained release tablets or their placebos at 7 a.m. and 5 p.m.

The alprenolol tablets had a disintegration time of 5 min according to the British Pharmacopoeia. The sustained release tablets released approximately 10% of the dose during the first hour and the rest continuously for 5 hours (7).

RESULTS

Fig. 1 shows the results of the trial comparing alprenolol and placebo after a long period of alprenolol treatment. All patients had more angina pectoris attacks on placebo than on the drug. Six out of nine patients were free from angina pectoris on treatment, but had chest pain in the placebo period. All patients considered the alprenolol period better.

In cases 5 and 6 the treatment period was repeated, and in cases 7 and 9 the placebo period, in order to evaluate the reproducibility of the test. The reproducibility was found to be qualitatively acceptable because cases 5 and 6 had no attacks in either of the alprenolol periods, while cases 7 and 9 had attacks of angina pectoris in placebo periods. The numerical agreement between the two placebo periods of the latter patients was, however, rather poor.

Fig. 2 shows the results of the comparison between alprenolol in ordinary tablet form and in sustained release tablets. Three patients had more attacks on alprenolol in ordinary tablets (cases 1, 5 and 7), while four patients had more attacks on the sustained release tablets (cases 2, 3, 8 and 9). Two patients had a similar number of attacks on both treatments (cases 4 and 6). There is no statistical difference between the effect of these two preparations.

Both trials thus included a period with alprenolol, 100 mg × 4 in the same patients. Approximately one year elapsed between the trials. The results, seen in Figs. 1 and 2 for individual patients, allow a comparison between alprenolol treatment in the same patients with approximately



Fig. 2. Number of attacks of angina pectoris per two weeks in individual patients in a double-blind cross-over trial of alprenolol in ordinary tablet form (100 mg 4 times daily) and in sustained release tablet (200 mg 2 times daily). Same patients as in Fig. 1. Trial performed one year after that in Fig. 1. White columns, sustained release tablets; black columns, ordinary tablets.

one-year interval. It is then seen that there is a considerable variation in number of attacks between the two times of testing.

Blood pressure showed no significant changes in the first or second trial.

In case 3 GOT was found to be 42 during treatment with sustained release tablets. He also admitted increased alcohol consumption during this period. Otherwise no pathological blood tests developed.

Case 3 reported a slight headache when taking sustained release tablets, and case 2 had a long period of chest pains giving rise to suspicion of myocardial infarction during the treatment with ordinary tablets in the second trial. During the placebo periods various minor complaints were reported such as anxiety (case 1), sleeplessness (case 7) and gastritis (case 6). Otherwise no observations possibly suspicious of side-effects were seen.

DISCUSSION

The results of the present investigation show that treatment of angina pectoris with beta-receptor blocking agents during a long period does not prevent recurrence of the symptom when the drug is withdrawn. Whether or not an improvement of angina pectoris would have occurred in these patients when they were without the drug, as compared with their original status before β-receptor blockade was introduced as treatment, is difficult to judge. The patients tested had all previously been tested with placebo after a run-in period (1, 2), and the total number of attacks during this initial placebo period was 120 during two weeks, while it was now 109. This difference is not statistically significant. No detailed conclusions from this result seem warranted, particularly because the spontaneous development of the angina pectoris of these patients is not known. *It can thus not be stated whether an increase in* physical activity which treated patients undoubtedly experience had any effect or not in these patients in terms of physical training. Physical training improves angina pectoris both subjectively and objectively (5, 6, 8). It may be concluded, however, that β-receptor blockade has an effect on angina pectoris after several years of treatment.

The ordinary tablet and a sustained release tablet of alprenolol showed no differences in effect, evaluated as angina pectoris attack frequency. Since placebo was found much less effective than alprenolol in the same patients, it seems possible to conclude that the sustained release tablet is effective and, in two doses a day gives the same protection from angina pectoris as ordinary tablets four times a day.

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LACTATE DEHYDROGENASE ISOENZYME PATTERNS IN SERUM AND SKELETAL MUSCLE IN INTOXICATED ALCOHOLICS

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Abstract. The lactate dehydrogenase (LDH) isoenzyme pattern in serum and skeletal muscle has been investigated in acutely intoxicated alcoholics. In serum a picture characterized by an elevation of LDH-1, LDH-2 and LDH-5 was most common. In skeletal muscle the alcoholics differed from control material with respect to the LDH isoenzyme distribution, with higher values for LDH-1 and LDH-2 and lower values for LDH-5.

An increase in serum transaminases (GOT and GPT) is common following an alcoholic debauch in chronic alcoholics. As abuse of alcohol is often accompanied by liver disease, it has usually been accepted that an increase in these enzymes should be connected with liver damage. There are, however, reports indicating that the skeletal muscle may be the source of an increase in serum enzymes following an alcoholic debauch. An acute muscle syndrome has been described in chronic alcoholics (16, 17). It has also been shown that an increase in the muscle specific creatinine phosphokinase (CPK) is about as common as an increase in GPT in chronic alcoholism (21).

Another serum enzyme known to increase following an alcoholic debauch is lactate dehydrogenase (LDH). Both liver and muscle tissue contain high levels of this enzyme. The enzyme may be separated by electrophoretic technique into five isoenzymes (LDH-1, LDH-2, LDH-3, LDH-4 and LDH-5 migrating farthest towards the anode) (27). The serum LDH isoenzyme pattern is often characteristic in disease roughly reflecting the isoenzymes of the damaged tissue.

The purpose of the present investigation was to study the serum LDH isoenzymes in chronic alcoholics during acute intoxication, in order to try to elucidate the nature of the increased serum levels. In addition an analysis of the isoenzyme pattern of skeletal muscle was made.

MATERIAL

The material comprised 49 chronic alcoholics (aged 23–64 years) consecutively admitted to an alcoholic ward. All of them were advanced abusers and were in a state of acute intoxication. Most had been treated earlier in hospital for alcoholism. None of the cases showed edema of the legs, ascites, or clinical evidence of cardiac decompensation. Electrocardiographic examinations, performed in connection with the skeletal muscle studies (see below), were normal except in one case (aged 64 years) showing an incomplete left bundle branch block. Two of the cases showed slightly lowered hemoglobin levels (12.6 and 12.8 g/100 ml). Six cases showed slightly increased bilirubin levels (1.1–1.9 mg/100 ml). None of the cases showed anhaptoglobinemia. In 15 of the cases (aged 23–64 years) additional studies of the LDH isoenzyme patterns of skeletal muscle were made. Similar studies were made on a control material comprising 17 healthy male medical students (aged 22–31 years).

METHODS

All blood samples for enzyme analyses were drawn in the morning on the day after admission to the hospital.

CPK was determined as described in the Sigma Technical Bulletin, no. 661 (22). Normal levels are given as 0–12 U/ml. In a control material, comprising 54 patients without any disease known to influence the serum CPK level, a range of values between 0–15 U/ml was found in this laboratory. Blood samples for CPK determinations in the control material were drawn in the morning on the day after admission to the hospital.

Serum GOT and GPT were determined by a kinetic UV method using reagents from AB Kabi, Stockholm, Sweden (20). Normal values for GOT 8–40 U/ml, and for GPT 4–35 U/ml.

All analyses of CPK, GOT and GPT were made on the day on which the sample had been drawn.

Total LDH was measured by an automatic fluorometric technique (14). Normal values 100–250 U/ml.

Determination of LDH isoenzymes was done by electrophoresis on agar gel (23). For normal values see Table I.

Determinations of total LDH and LDH isoenzymes were usually done on the day on which the sample had been

Table I. *Serum LDH isoenzyme activities in healthy controls and in acutely intoxicated alcoholics*

U ml	Healthy controls (44) Mean	Mean ± SD	Alcoholics (=49) Mean	Mean ± 2 S.D
LDH 1	79	48–110	132	70–202
LDH–2	59	30–88	105	55–155
LDH–3	21	8–34	43	11–75
LDH–4	8	3–13	20	1–49
LDH 5	6	10	30	0–84

drawn. Otherwise the samples were stored at room temperature for at most 4 hours.

Muscle biopsies. The biopsy was performed with muscle biopsy needle in the lateral quadriceps muscle as described by Bergström (4). The weights of the biopsy specimens, hich varied between 5–30 mg, were obtained by extrapolation from the weights recorded at ., 3 4 and 5 min after the biopsy had been performed. The specimens were homogenized in ml aq. dest. using small Potter-Elvehjem glass homogenizers.

The homogenates were placed in refrigerator at −20°C for 10 min before centrifugation. This was found to produce maximal yields of the arious isoenzymes. Controls made on cell-free muscle extracts showed that the quick freezing did not significantly affect either the total LDH or the isoenzyme distribution. The samples were analysed immediately or stored at room temperature for at most 4 h. Before electrophoresis 0.1 ml 20% bovine albumin in 4.5% NaCl was added to 0.4 ml muscle extract.

In order to check the reproducibility of the determination of the LDH isoenzyme distribution in the quadriceps muscle of the same indi idual, and at the same time to check the analytical procedure, second biopsy was performed one week later in the contralateral quadriceps muscle in eight healthy controls. The error of a single determination f the percentage distribution of the isoenzymes was then calculated as $\pm\sqrt{\Sigma d^2/2n}$ (8), here d is the difference between the t observed percentage alues and is the number of duplicate determinations. The following values were obtained for the five isoenzymes. LDH 1 ±0.9 LDH ±3.4 LDH-3 ±5.3, LDH-4 ±6.3 LDH 5 ±11

The corresponding values for the LDH isoenzyme activities, calculated as units per mg wet weight muscle were: LDH-1 ±1.3 LDH 2 ±7.9 LDH-3 ± 1 LDH-4 ±22, LDH-5 ±54

Statistics. Ordinary standard methods ere used. The rank correlation method (11) was used to estimate the degree of correlation, since the nature of some of the experimental data rendered treatment by ordinary methods less convenient.

RESULTS

Serum total LDH and LDH isoenzymes

Table I shows results obtained in the material of alcoholics as well as in a control material of healthy persons. Of the 49 alcoholics 39 displayed increased total LDH activities. The ten cases with

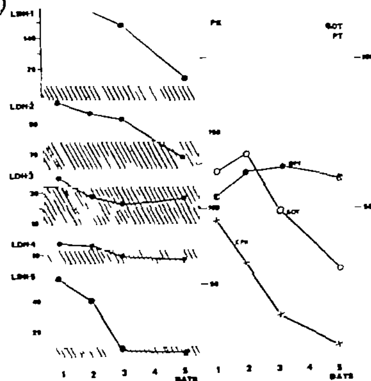


Fig. 1. Serum LDH isoenzyme pattern in a case with elevated serum CPK, GOT and GPT indicating liver and muscle injury. In this case, LDH-1 LDH- and LDH 5 were increased.

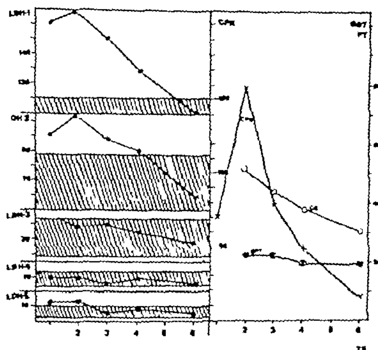


Fig. Serum LDH isoenzyme picture in patient with muscle enzyme pattern (elevated CPK and GOT but normal GPT). In this case LDH-1 and LDH 2 are increased.

normal total LDH showed normal LDH isoenzyme distribution. In most of the cases (31 cases) with elevated total LDH, an increase in LDH 1 LDH-2 and LDH 5 dominated the pattern, as illustrated by the case given in Fig. 1 In this case the serum CPK, GOT and GPT activities were increased. In five other cases with the same LDH isoenzyme picture (elevated LDH 1 LDH 2 and LDH 5), daily serum determinations for three to ten days revealed elevated serum GOT and GPT activities in all five cases, and CPK was found to be increased in all but one case

A serum LDH isoenzyme pattern characterized by an increase of LDH 1 and/or LDH 2 without any essential elevation of LDH 5 was found in eight cases. A typical case is shown in Fig. – As seen, the serum CPK and GOT but not the GPT activities, were increased. Four additional cases with the above LDH isoenzyme pattern were followed for three to nine days. Three of them showed an enzyme pattern similar to that of the case illustrated in Fig. – In one case however the serum GPT activity was also increased (55 U/ml).

The cases with high LDH 5 also showed proportionally higher GPT levels (Fig. 1) A rank correlation test revealed a significant correlation between GPT and LDH 5 ($p < 0.001$) LDH-4

Table II. *Skeletal muscle isoenzyme distribution in healthy controls and in acutely intoxicated alcoholics*

Per cent	Healthy controls ($n = 17$)		Alcoholics ($n = 15$)		Significance of differences between means
	Mean	Range	Mean	Range	
LDH-1	2.4	0–7.9	6.8	0–34	$p < 0.001$
LDH-2	8.1	0.6–21	18	0.9–39	$p < 0.001$
LDH-3	19	3.7–33	27	9–43	$p < 0.001$
LDH-4	20	4.9–35	12	8–79	—
LDH-5	50	23–80	25	10–70	$p < 0.01$

Table III. *Skeletal muscle isoenzyme activities per mg wet weight in healthy controls and in acutely intoxicated alcoholics*

U/mg	Healthy controls ($n = 13$)		Alcoholics ($n = 9$)		Significance of differences between means
	Mean	Range	Mean	Range	
Total LDH	226	35–630	126	51 320	—
LDH-1	5	1 17	7.7	2 16	—
LDH 2	16	1–6.	23	11–50	—
LDH 3	38	6–125	37	17–82	—
LDH-4	50	11–149	27	6–70	—
LDH-5	115	15–277	31	5–114	$p < 0.05$

Table IV. *Correlation between the LDH isoenzymes and CPK, GOT and GPT in 49 alcoholics*

Rank correlation coefficients given

	LDH-1	LDH-2	LDH-3	LDH-4	LDH-5
CPK	0.[illegible] $p<0.05$	0.25	0.[illegible]	0.11	−0.[illegible]
GOT	0.[illegible] $p<0.001$	0.4[illegible] $p<0.01$	0.5[illegible] $p<0.001$	0.54 $p<0.001$	0.67 $p<0.001$
GPT	0.[illegible]	0.[illegible]	0.32 $p<0.05$	0.41 $p<0.01$	0.59 $p<0.001$

($p<0.01$) and LDH-3 ($p<0.05$). Cases with high LDH-1 and LDH-2 levels tended to show also high CPK levels (Fig. 2). A significant correlation was found between CPK and LDH-1 ($p<0.05$). GOT, finally, was correlated to all the LDH isoenzyme fractions (Table IV).

LDH-1/LDH-2 ratio

The mean serum LDH-1/LDH-2 ratio in the 39 alcoholics with elevated total LDH activities (250–630 U/ml) was 1.31 ± 0.04 (Mean ± S.E.), i.e. close to the value found for healthy persons (1.36 ± 0.03, $n=44$). For comparison, this ratio was determined in 1[illegible] cases of myocardial infarction with a similar range of serum total LDH levels (250–700 U/ml). The mean ratio of 1.89 ± 0.13 was significantly higher ($p<0.001$) than the ratio observed in the alcoholic material.

Specificity of the serum LDH isoenzyme changes

In order to elucidate the specificity of the type of LDH isoenzyme change shown above to be common in alcoholics, a survey was made of 1 000 consecutive LDH isoenzyme electrophoreses, performed routinely in connection with protein electrophoresis. Of 185 adult in-patients, with total LDH in the same range as in the present series of alcoholics (i.e. 250–700 U/ml), 30 showed a pattern characterized by an elevation of LDH-1, LDH-2 and LDH-5. Fourteen of these cases presented a history of advanced alcoholic abuse. The common diagnoses of the other patients with LDH-1, LDH-2 and LDH-5 elevation was that of diseases of the liver and/or biliary tract apparently unrelated to alcoholic abuse (5). The remaining diagnoses were: sepsis (2 cases), miliary tuberculosis (1 case), femoral vein thrombosis (1 case), intervertebral disc hernia (1 case), pancreatic disease (1 case), hemolytic disease (1 case), decompensatio cordis (1 case), acute cerebral vascular accident (1 case). In two cases, finally, there was proximal weakness in the legs, in one case due to hypokalemia and in the other of unknown etiology.

Distribution of LDH isoenzymes in skeletal muscle

The distribution of the LDH isoenzymes in the muscle biopsies is shown in Table II. The alcoholics differed from the control material with respect to the isoenzyme distribution, with high values for LDH-1 and LDH-2 and low values for LDH-5. The total LDH activities (Table III), as measured in U/mg wet weight of the biopsies, varied within wide limits (35–630 U) both in the alcoholic and in the control material. There was no significant difference between the mean total LDH activities in the two series. Nor was there any significant difference between the mean total activities for LDH-1, LDH-2, LDH-3 and LDH-4 in the two groups. However, the mean total activity for LDH-5 was significantly lower in the alcoholics than in the healthy individuals ($p<0.05$).

DISCUSSION

The investigation has shown that in chronic alcoholics, following a period of high alcohol abuse, all LDH isoenzymes in serum are increased in most cases, with a predominance for LDH-1, LDH-2 and LDH-5. This pattern seems to be rather characteristic for chronic alcoholics.

An elevation of serum LDH-5 has been reported mainly in liver disease (3). Liver disease of all grades is one of the hallmarks of chronic alcoholism, and in this series a significant correlation was found between LDH-5 and GPT, supporting the idea that the LDH-5 elevation might be due to liver damage. There was no correlation between CPK and LDH-5, and thus no support for the idea that skeletal muscle, known to contain predominantly LDH-5 (1[illegible]), was the source of the increased serum LDH-5 in the alcoholics. An elevation of LDH-1 and LDH-2 is known to occur in hemolytic disease (3). In this connection it should be mentioned that increased hemolysis has been reported in alcoholics with Zieve's syndrome as well as with advanced liver cirrhosis (18, 19, [illegible]). None of the patients in the present series showed clinical or laboratory

findings consistent with Zieve's syndrom and/or advanced liver cirrhosis. Anhaptoglobinemia was not observed in any of the cases. Thus it is not likely that the increased levels of LDH 1 and LDH 2 were due to hemolytic disease.

An elevation of LDH 1 and LDH 2 has earlier been reported in heart disease (3). Cardiac disease has been described in chronic alcoholics (1 6 15). However in the present series of alcoholics the case histories and available clinical data gave no indication that the enzyme changes were related to cardiac disease. Further the mean value for the LDH 1/LDH 2 ratio was significantly lower in the alcoholics than the mean ratio found in patients with myocardial infarction. This, of course, does not necessarily exclude myocardial muscle injury as a source of the LDH 1 and LDH increase in the alcoholics.

In most normal human skeletal muscles LDH 5 is predominant. However it is clear that different muscles vary greatly in their LDH isoenzyme distribution (24). Thus, for instance, the soleus muscle is reported to contain mainly the fast LDH isoenzyme fractions (9 4) Further it has been shown that the muscles in myopathy or neurogenic atrophy often show a low relative content of LDH 5 with increased proportions of LDH 1 and LDH 2 isoenzymes (7 13 25). This change in the muscle isoenzymes has been considered to contribute to the serum elevation of LDH 1 and LDH 2 in muscular dystrophy (26) In the alcoholics a change of the skeletal muscle pattern in the same direction as reported in myopathy and neurogenic muscle disease was observed (Tables II and III). Accordingly it does not seem unlikely that the serum increase in LDH 1 and LDH 2 observed in the alcoholics reflects the skeletal muscle changes. Following a release of LDH from the muscles an accumulation in serum of the fast isoenzymes may occur having regard to the much lower rate of elimination from serum of fast than of slow LDH isoenzymes (5 10).

The cases illustrated in Figs. 2 and 3 as well as the other nine cases followed with daily serum enzyme determinations, show that LDH 1 and LDH elevation mostly occurs together with an elevation of CPK, while a LDH 5 increase usually is connected with GPT levation. A significant correlation was also found between CPK and LDH 1 ($p < 0.05$). A strong correlation between CPK and LDH 1 is not to be expected, considering that CPK returns to normal levels much earlier than LDH 1 after muscle damage (3). GOT which is known to increase in liver as well as in skeletal muscle disease, was correlated to all the LDH isoenzyme fractions. These findings are consistent with the idea that the LDH 1 and LDH 2 isoenzymes arise from skeletal muscle and that the LDH 5 increase derives from the liver

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THE DISTRIBUTION OF PROTEINS BETWEEN INTRA AND EXTRAVASCULAR SPACES IN HEALTH AND DISEASE

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Abstract. The extravascular distribution of albumin and immunoglobulin G has been studied with special regard to the effect of prolonged bedrest. The EV/IV ratio of these proteins is very much influenced in the conditions studied. The exact effect of posture could not be obtained in this study but the results should inspire caution in judging the results of metabolic studies in patients confined to bed in relation to controls. Some errors in retained dose measurements have been pointed out. Although the mechanism is unknown, it has been shown that the EV/IV regulation of proteins has been influenced by external means in severely burnt patients.

One special advantage in studies of protein catabolism in health and disease with the aid of radioactively labelled proteins is that they offer a means of estimating the amount of the protein that is localized outside the vascular compartment and is in exchange equilibrium with it. This can either be done indirectly by mathematical analysis of the plasma activity curve (10) or directly by measurements of plasma activity and total excreted activity in urine and faeces (6, 12). The most direct and accurate way of determining the radioactivity in a patient is by means of a whole body counter.

In studies of protein catabolism in health and disease patients have been observed with extravascular pools (EV) unusually large in relation to the intravascular pool (IV) (2, 4). This relation is best expressed as the EV/IV ratio. For IgG for example, this ratio is virtually 1 in our healthy control material with a standard deviation of only 0.14. Thus observed values of 2–3 for the EV/IV quotient were really far from normal, and it seemed worthwhile to investigate the conditions and reasons for such changes in distribution. Often the high EV/IV quotients were observed in patients who had been subjected to a long period of bed rest.

In order to investigate this problem further we studied a group of nine patients with cerebral injuries who had been confined to bed for a long time. The patients were injected with gammaglobulin tagged with ^{131}I, which permitted us to perform whole-body counting. We have previously shown that some activity is lost from the body by other routes than urine and faeces (1). Thus we felt that whole-body counting was necessary to obtain reliable measures of the extravascular pool. The retained dose was, however, also obtained by urine counting, but the results to be reported here refer to the wholebody measurements.

METHODS

IgG-globulin was prepared from pooled normal sera by chromatography on DEAE-Sephadex followed by gel filtration on Sephadex G-200. Phosphate buffer 0.05 M pH 8.0, was used.

Labelling with ^{131}I was done by McFarlane method (8). The labelled protein preparation was tested for sterility and pyrogens.

Quantitative determination of IgG-globulin in serum was done by 1) determination of the gammaglobulin fraction by (a) electrophoresis and (b) free boundary electrophoresis by the Tiselius method; 2) the single radial diffusion method described by Mancini et al. (9). Total serum-protein values were determined by biuret method. Comparisons were made on means of repeated samples.

Performance of the isotope studies

About 20 μCi ^{131}I-labelled IgG-globulin (equalling at the most a few mg of IgG-globulin) or albumin was injected intravenously. Blood samples were taken 10 min after the injection, and then daily for two weeks, thereafter every second to third day. The plasma volume was calculated by the isotope-dilution technique. The subjects were carefully instructed to save all the urine for each 4-hour

Table I *Distribution of ^{131}I IgG on different days in immobile patients and controls as obtained from whole-body counting*

Day	Group	EV/IV ± S D
5	Patients (9)	1.22 ± 0.19
	Controls (7)	0.68 ± 0 09
25	Patients (6)	1.96 ± 0.50
	Controls (7)	0.93 ± 0.14
38	Patients (4)	1 71 ± 0.46
	Controls (7)	0.99 ± 0.14

period. From these amounts samples ere taken for measurements of radioactivity. Usually the urinary activity could not be measured ith satisfactory precision after 25 days.

Whole-body counting was done one and three hours after the injection of the isotope and then simultaneously with the blood sampling.

In all cases sodium-iodide solution (10 drops of 10% NaI daily) was *given three days before and throughout* the period of investigation, which covered 50 days.

Methods for calculation f metabolic data

Mathematical analyses f the plasma-activity curv was done by Matthews method (10). In addition the 4-hour urinary excretion of activity (U) as divided by the mean activity in plasma during 4 h (P). The resulting ratio U/P is measure of the catabolism and is constant throughout the period of study if the breakdow occurs in the intravascular pool or in a compartment ith rapid exchange ith the intravascular space.

The excretion of activity as obtained also from the differences between successive hole-body counts. The ratio of the difference between tw successive whole-body counts to the plasma activity can be used to obtain measure of the catabolism. The calculations were made with digital computer with an ALGOL programme PLASMA (11). All U/P and whole-body calculations ere performed on measurements between days 5 and 26

Methods for determination of hole-body activity

The patient lies supine on a stretcher in a steel-walled room. One scintillation detector with a 5 -sodium-iodide crystal moves above and another below the stretcher. All pulses over 0.1 Mev were counted in order to obtain great sensitivity. The patient's background activity was measured before injection of the isotope. The efficiency of measurement of ^{131}I was about 1%.

Measurement f radioactivity

Four ml plasma or urine were measured in well scintillation counter. Only the activity in the photopeak at 0.36 Mev for ^{131}I was counted.

Definitions

Catabolism. The normal or pathological process by which the molecules of substance are converted to other molecules or ions.

Fractional catabolic rate. Catabolism per unit of time xpressed as a percentage of the intravascular pool. If the size of this pool is known, the catabolism can be expressed in g per day.

Retained dose. The body total activity calculated by subtracting the total urinary and faecal excretion of activity from the given dose.

Whole-body activity. The body's total activity measured ith a whole-body counter.

Pool. The area or the form in which a substance occurs. A pool may be a physiologically or chemically defined form. The possibility of defining pool is dependent on its rate of xchange with other pools in relation to sampling intervals and the time of investigation.

EV/IV ratio. When the EV/IV ratio is reported, it is essential to specify at what time after injection of the tracer the ratio is obtained and how it is calculated. This is further discussed below. W have found that in steady state patients the EV/IV ratio reaches constant alue after about three weeks. EV/IV ratios in this paper are reported as the mean of 3–5 measurements around that time, unless otherwise specified. The EV pool is obtained by subtracting the plasma pool activity from the whole-body activity.

RESULTS AND DISCUSSION

Table I shows the EV/IV ratios obtained on different days after injection. The mean EV/IV ratios in the patients are about twice the ratios in the controls. The difference is statistically highly

Table II. *Comparison between gammaglobulin pool sizes and catabolism in immobile patients and controls. Albumin pool shown for comparison*

Group	Plasma vol. (l)	IV IgG (g/100 ml)	IV IgG (pool, g)	IV albumin (pool, g)	IgG catabolism (U/P) (/24 h)	(mg/kg 4 h)
Nine pats. with cerebral lesion	2.19 ± 0.28	1.31 ± 0.45	33.9 ± 13.6	83.5 ± 15.2	8.1 ± 2.3	30 0 ± 14.7
Controls	3.10 ± 0 45	0.99 ± 0.13	28.6 ± 6.8	127.9 ± 21.3	4 7 ± 1 0	18.7 ± 4.9
No. of controls	15	7	7	15	7	7
Significance			—			

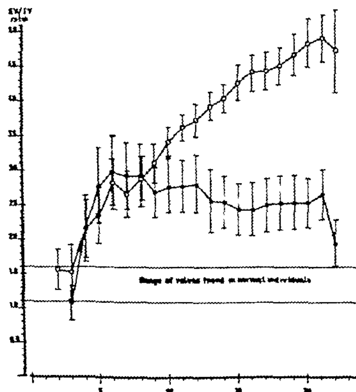


Fig. 1. Change in the EV/IV ratio for labelled albumin injected in severe burns. On the abscissa the time in days, on the ordinate the EV/IV ratio. ○ patients treated by the exposure method at room temperature (22°C). ● patients treated in warm dry air (32°C). Mean and S.D. shown. Both groups comprised about 15 patients with burns between 25–60% of the body surface.

significant. In these patients the ratio does not change appreciably during the investigation period.

An interesting point is that, in spite of this altered distribution ratio the plasma pool of IgG is unaltered. The plasma volume is significantly lower but a higher IgG concentration maintains the intravascular pool size, as seen in Table II.

One reason for the increased plasma IgG concentration may be that all patients had urinary tract infections owing to the catheters à demeure which had to be used.

The catabolic rate is also much increased over the control values. This is remarkable, as we have previously shown that a highly significant correlation exists between the amount of IgG in the plasma pool and its catabolic rate (3). This further supports the assumption that the catabolism is regulated by the synthesis rather than by the circulating IgG as suggested by Waldman and Schwab (13).

Speculations concerning the results from this group of patients should, of course, only be made with great caution, as all patients suffered from cerebral vascular disease (cerebral haemorrhage or thrombosis). We cannot be certain that all deviations from normal observed by us were due solely to the changed posture and immobility of the patients. The disease itself may also have influenced the results. In order to obtain additional information, two other groups of patients were observed in collaboration with Liljedahl, Davies and Reizenstein (5). One was a group of paraplegic patients, the other one consisted of patients with severe burns.

High EV/IV ratios were obtained in these patients for both proteins. Fig. 1 shows how the EV/IV ratio for albumin changes in severely burnt patients. The mean and standard deviation are indicated. The two horizontal lines are the limits for this ratio in control cases. The open circles show the EV/IV ratios in patients with between 25 and 60% third-degree burns treated at normal room temperature (22°C) by the exposure method. As will be seen, the ratio increases during 3 weeks to about 5. The filled circles refer to patients with the same magnitude of burn, also treated by exposure but in a stream of warm dry air. The air is distributed through the per-

Table III. IV *pool size catabolism and distribution of albumin in five paraplegic patients*

Case	Plasma albumin (g/100 ml)	(pool, g)	Catabolism (U/P) (./24 h)	EV/IV
M. Q	3.1	107	17 8	4.03
N S.	3.2	86	18.7	3.14
O A	3.6	91	17.3	4 70
O Y	3 6	133	12.1	2.22
P V	3 6	120	9.9	2.00
Mean				3.22
EV/IV range in controls				1 1 1.6

forated bottom of the bed and through the poly ether mattress and thus surrounds the patients with air at a temperature of about 32 C (7) This treatment apparently influences the EV IV ratio very greatly In the case of severe burns we know that the distribution depends very much upon the characteristics of the injury Fig. 1 shows how the distribution can be affected by external means. This will be discussed later

The same type of study with [131]I albumin was performed on five paraplegic patients. These patients had been injured and confined to bed for two months or more before the injection of the labelled albumin. The operation was not the same in all cases, but was a major surgical procedure

Table III shows how albumin is distributed and catabolized in these patients. Again we find very high EV/IV ratios. The catabolic rate is high and the intravascular pool is normal or lowered. This group is small and not homogenous, but common to these patients is their paralysed condition and the abnormal EV/IV albumin ratios.

The reason or rather reasons for the high EV/IV ratios for IgG and albumin distribution observed by us in these three patient groups are not known with certainty They probably differ somewhat, moreover in the different groups. A known fact is that changing from upright to supine position lowers the plasma volume by about 10%, most probably due to the altered pressure relation between intra- and extravascular spaces.

It is not plausible that this pressure effect alone would even in the long run, lead to the high EV/IV ratios reported here. Common to the group with cerebral lesion and the paraplegics is that they are motionless. Thus the lymph transport must be very slow indeed and this may be a major factor in causing the increasing extravascular pool In contrast to this immobility the burn patients can and do move in different ways. Especially the patients treated at room temperature shiver in an attempt by the body to compensate their big heat losses. These patients, however show the highest observed EV/IV ratios. On the other hand they have capillary injuries, which probably also increase the extravascular pool.

The low albumin concentration in these patients implies a low colloid osmotic pressure, which also may influence the distribution and lead to an increase of the EV pool.

Some possible reasons for an increased EV/IV ratio are: 1) impaired lymph flow: 2) capillary injury: 3) increased capillary permeability: 4) low intravenous colloid osmotic pressure: and 5) altered pressure relationship between extra and intravascular spaces. In addition to these there may be others. It is, for instance, not easy to understand how the warming of the burn patients influences this ratio in a normalizing direction. This was elaborated by Birke et al. (5). We may speculate about another factor that must be considered in these patients. Following the burn injury the rate of albumin distribution from the intravascular to the extravascular pool increases very much and is not fully compensated by the

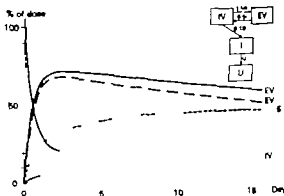


Fig Compartment model and analogue computer solution to the study of the iodide excretion pool and its effect on the EV/IV calculation. IV intravascular pool, EV extravascular pool, I, iodide pool, U excretion pool. The figures denote the fraction of the pool transferred in indicated direction per 24 h. The value of the iodide pool is also shown multiplied by 5

reverse distribution. This leads to an increase of the extravascular pool. Five to eight days after the IV to EV distribution rate decreases, but again the reverse change lags behind. This leads to some restoration of the IV pool, which can be observed in Fig. 1. The EV/IV curve for the patients treated at room temperature shows an inflection at that time and even a minute decline before it again increases.

In the warm-air-treated patients the increase in the EV/IV ratio is stopped at the same time and remains at the level reached. If no protein therapy is given to severely burnt patients, both albumin and gammaglobulins virtually disappear from the plasma pool. All these patients were, however given adequate albumin infusions to keep the serum albumin level almost normal. But, of course, the labelled albumin is rapidly moved to the EV pool.

As the intravascular albumin pool is maintained at an almost normal level in these patients, this may delay the redistribution of the big EV albumin pool to the IV pool.

Thus the main part of the labelled albumin will be, as it was, trapped in the EV pool and, as we are measuring the radioactivity and not specific activity we obtain big EV/IV ratios. The point is that these high ratios will be somewhat misleading if we do not clearly realize that we are in fact measuring what happened to the plasma pool that existed when we injected the labelled albumin. This has been elaborated previously (4).

A comment also on the technique for establishing the size of the extravascular pool. This can be achieved in principle by two methods. One is by *counting all the activity excreted in the urine* and subtracting from the dose administered. This gives what is usually called the retained dose. From this is subtracted the plasma pool activity to give the total extravascular pool. The other way is direct whole-body counting, which gives the whole-body activity from which the plasma pool activity is subtracted, as before, to give the extravascular pool. In prolonged studies with I-tagged IgG we found an increasing difference between the retained dose and the whole-body activity (1). W could not explain this in any other way than as a loss of radioactivity by other routes than urine and faeces. This difference was found to be 9.8% of the dose after 25 days. The whole-body activity is thus 9.8% lower than the retained dose. The other routes than faeces and urine are assumed to be sweat, saliva and, in the case of burns, wound exudation. Further we assume that this loss occurs from the intermediate iodide excretion pool. This would imply that, in cases where this pool is large, the losses could be much greater than the figure given. Thus very erroneous estimations of the extravascular pool may result and are further accentuated when the EV/IV ratio is calculated. As an example may be mentioned a burns case in which the EV/IV ratio calculated from the retained dose was 11.0. From the whole-body measurements the ratio 2.1 was obtained. This is not difficult to understand, as wound losses are not accounted for by retained dose measurements but are automatically obtained in the whole-body counting.

The iodide pool could be expected to be greater in the paralysed patients. This was investigated by injecting the cerebral lesion patients with ^{131}I iodide and measuring the urinary excretion which usually amounts to 50% of the body pool per 4 hours in control cases. In these patients it varied between normal and 70% of the body pool per 24 hours. This does, of course, affect the measurement of the EV pool.

In order to estimate the magnitude of the delayed iodide excretion, this situation was simulated on an analogue computer.

Fig. 2 shows the compartment system used for this study, the distribution parameters applied and the resulting curves.

The overestimation of the EV/IV ratio is around 16% a few days after injection, and changes very little throughout the following period. *It is about 17% three weeks after the injection*, and this figure is thus an estimate of the error caused by slow iodide excretion which might exist in the reported EV/IV figures.

ACKNOWLEDGEMENTS

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Mean				3
EV/IV range in controls				1

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METHOXAMINE INJECTIONS IN THE DIAGNOSIS OF MITRAL INSUFFICIENCY DURING ROUTINE RIGHT HEART CATHETERIZATIONS

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Abstract. The effect of methoxamine on PCV pressure has been examined in 47 cases (24 with mitral valvular disease, 2 with aortic insufficiency, 12 with congenital heart disease, and 9 with no organic heart disease). The rise in v-peak pressure related to the elevation in systemic arterial pressure (Δ /ΔA ratio) after methoxamine injection was measured. The ratio usually was higher in patients with mitral insufficiency than in other cases, but marked overlapping was seen between the different patient groups. The methoxamine test during routine cardiac catheterization is therefore of limited value in the diagnosis of mitral insufficiency. A marked bradycardia was seen after methoxamine infusions in most cases. Four patients had transient conduction disturbances, but no serious complications were encountered. The effect of methoxamine on cardiac output was recorded in 13 cases. Methoxamine did not influence cardiac output significantly.

In cardiac catheterization the verification of mitral insufficiency has to be based on a left ventricle angiogram. Even if the pressure curves obtained from the left atrium or in the pulmonary wedge position often are characteristic, with high v-peaks and a high Ry/v ratio, a diagnosis based on these curves may be wrong, many cases with mitral insufficiency will be overlooked, and some erroneously diagnosed. An alternative method for the diagnosis of mitral insufficiency therefore will be of great value.

Mitral regurgitant flow according to Gorlin et al. (4), will depend on the mitral regurgitant area, systolic ejection period and the systolic pressure gradient between left ventricle and left atrium. After a rise in systemic pressure the pressure gradient will increase, and thus, if the other factors are unchanged, mitral regurgitant flow will increase.

Braunwald et al. (2) found that, during infusion of pressor-amines, the rise of v-peak pressure in the left atrium was significantly higher in patients with mitral insufficiency than in those without.

Methoxamine, which has a pure vasopressor activity without positive inotropic effect on the ventricular musculature (2, 3), seems to be the drug of choice when an isolated blood pressure elevation is wanted. It has been used in the diagnosis of mitral insufficiency during phonocardiography (8) and after acute myocardial infarction (5).

The purpose of the present investigation has been to see whether the registration of PCV pressures after the administration of methoxamine during routine cardiac catheterizations could be of value in the diagnosis of mitral insufficiency.

MATERIAL AND METHODS

Forty-seven patients were examined, twenty-four with mitral valvular disease, two with aortic disease, twelve with congenital heart disease, and nine with no organic heart disease.

The nine patients examined with no organic heart disease all had diagnostic heart catheterization. Seven had physiologic murmurs, one funnel chest, and one moderate pulmonary fibrosis.

The patients examined were admitted for routine diagnostic cardiac catheterization. Patients with systolic blood pressure more than 150 mmHg and patients with incompensated cardiac insufficiency were excluded from the study. In all patients with mitral valve disease, left ventricle angiogram has been performed.

After a Cournand catheter had been placed in PCV position and a polyethylene catheter in the aorta or the left ventricle, methoxamine (Vasoxine, Burroughs Wellcome) diluted in physiological saline to 1 mg/ml was injected in the right heart catheter at a rate of 2 mg every minute. PCV pressure and systemic arterial pressure (or left ventricular pressure) were recorded every two minutes. For pressure calculations the average of three successive heart beats was used. The injections were stopped when

Table I. *The effect of methoxamine on the PCV pressure curve. The PCV mean pressure and v-peak pressure recorded shortly before methoxamine injection and the highest pressures recorded after the injection. The rise in v-peak pressure related to the rise in systemic arterial pressure (Δv/ΔA ratio) after methoxamine injection*

MI = mitral insufficiency. MS = mitral stenosis. AI = aortic insufficiency.
ASD = atrial septal defect. VSD = ventricular septal defect.
+ = slight. + + = moderate. + + + = severe.

Diagnosis	v-peak (mmHg)		PCV mean pressure (mmHg)		Δv/ΔA [a]
	Before	After	Before	After	
MI	45	76	16	22	
MI +	11	37	5	12	225
MI	10	14	8	10	[illegible]3
MI + + MS + AI	15	27	10	18	17
MI + MS + + AI + +	17	3	10	18	50
MI MS AI +	1	29	8	18	47
MI MS AI	38	6[illegible]	26	31	300
MI MS +	18	31	13	17	41
MI MS	29	34	19	20	14
MI + MS +	15	4	10	15	14
MI + MS +	27	25	17	18	2
MI MS AI +	33	60	22	40	27
MI + MS +	17	28	10	18	21
MI + + MS +	30	33	17	19	9
MI + MS +	30	3[illegible]	20	22	5
MS AI	10	18	9	13	2[illegible]
MS + AI +	13	38	10	19	56
MS	12	37	9	22	26
MS +	16	15	12	13	0
MS +	8	19	5	15	13
MS	28	30	20	20	10
MS +	1	19	7	13	22
MS	17	1	13	14	8
MS	32	34	26	27	11
AI + +	6	20	5	16	49
AI + +	16	47	11	34	650
ASD (average from 11 pats.)	7	13	4	8	14
VSD (1 pat.)	12	18	7	14	17
No organic heart disease (average from 9 pats.)	5	1	4	8	16

[a] rise in systemic pressure

ystemic arterial pressure had been elevated 50 mmHg or more or after a total dose of 20 mg methoxamine. In 13 patients the right heart catheter was withdrawn to the pulmonary artery 5 min after the start of methoxamine injections and cardiac output was measured.

RESULTS

The effect of methoxamine on the PCV pressures appears from Table I. Usually the greatest effect after methoxamine injection was seen during the first 2–4 min. Later on, the PCV curve often flattened out and the difference between v-peak and PCV mean pressures was reduced. The increase in v-pressure is related to the rise in systemic arterial pressure (A). Therefore the ratio Δv/ΔA will be the most exact parameter of the methoxamine effect on mitral insufficiency. In the present investigation this ratio was on an average more than 100% in patients with pure mitral insufficiency, 59% in patients with mitral stenosis and insufficiency, and 17% in patients with mitral stenosis only. A marked overlapping was seen between the groups. High Δv/ΔA ratios were encountered in two patients with severe pure mitral insufficiency, while one patient with slight pure mitral insufficiency had a low ratio. High Δv/ΔA ratios were also seen in 3 of 13 patients with combined mitral stenosis and mitral insufficiency. A marked mitral insufficiency was seen in all these three cases, but also in six other patients in whom the Δv/ΔA ratio was not elevated. Two patients with an isolated aortic insufficiency

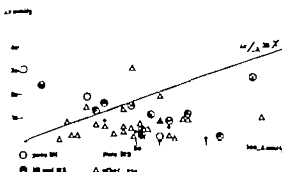


Fig. 1 Effect of methoxamine injection on v-peak pressure. Rise in v-peak pressure (Δ) related to rise in systemic arterial pressure (ΔA).

also had a markedly elevated Δv/ΔA ratio. Eleven patients with atrial septal defects all had normal ratio (average 14%). One patient with ventricular septal defect had 17% and in nine without organic heart disease the average ratio was 15%

In Fig. 1 Δv/ΔA is illustrated. The dotted line represents a Δv/ΔA ratio of 35% which is estimated to be the upper normal limit (7).

In this patient material including cases with both mitral stenosis and mitral insufficiency the calculation of Δv/ΔA after the injection of methoxamine was therefore of only minor value in the selection of patients with mitral insufficiency

The average value of the Ry/v v/PCV mean pressure and v–PCV mean pressure in different patient groups appears from Table II. Ry/v fell after methoxamine injection in all patient groups. In patients with pure mitral insufficiency Ry/v was high before and lower after methoxamine injection. The ratio v/PCV mean pressure increased after methoxamine injection in 9 of 15 patients with mitral insufficiency in 4 of 7 patients with pure mitral stenosis, and in 10 of 25 of the other patients. The difference v–PCV mean pressure increased after methoxamine in 13 of 15 patients with mitral insufficiency in 6 of 7 patients with pure mitral stenosis, and in ?0 of the 25 other patients. Thus the calculation of these parameters in relation to methoxamine injections seems to be of no value.

Cardiac output was calculated before and after methoxamine injection in 13 patients (Table III). Methoxamine did not change cardiac output significantly In six patients CO increased, in five fell, and in two remained unchanged. Stroke volume fell in three, was unchanged in one and increased in nine patients. The increase in stroke volume was associated with the reflex bradycardia induced by methoxamine. Pulmonary arterial resistance increased considerably in two patients with mitral insufficiency in both these patients a significant increase in the Δv/ΔA ratio had also been observed. In the other 11 patients without mitral insufficiency no change was noted.

The rise of systolic blood pressure started during the first two minutes after the injection of methoxamine and the highest blood pressure was usually reached within 4–6 min. The pressor response was less pronounced in patients with poor cardiac function (function groups III and IV) than in the other cases. The average increase in blood pressure in different patient groups appears from Table IV

A fall in heart rate after methoxamine injection was observed in all patients except two On an average the heart rate was reduced to 67 of the preinjection rate. The fall in heart rate

Table II. *The effect of methoxamine injection on the PCV pressure curve Average values for Ry/ PCV and v/PCV in different patient groups before and after methoxamine*

Diagnosis	No of pats.	Ry/v Before	Ry/v After	v–PCV Before	v–PCV After	/PCV Before	/PCV After
MI, pure	3	3.2	2.9	12	24	2.1	.4
MI + MS	12	1.5	1.2	8	14	1.6	1.6
MI, all cases	15	1.9	1.5	9	16	1 7	1.8
MS, pure	7	1.5	0 9	6	7	1 7	1.5
AI, pure	2	2.2	2.1	3	9	1 4	1 4
ASD	11	2.3	1 7	3	5	1.6	1.5
VSD	1	3 8	1.2	5	4	1 7	1.3
No organic heart disease	9	1.8	1.8	2	4		7

Table III *Cardiac output (CO), stroke volume and pulmonary arterial resistance (PAR) after methoxamine*

Diagnosis	Age	CO Before	CO After	CI Before	CI After	Stroke volume (ml) Before	Stroke volume (ml) After	PAR (Dyn sec. cm) Before	PAR (Dyn sec. cm) After
Normal	48	4 9	5 4	3.0	3.3	68	84	—	133
MI	59	5.5	4 1	[illegible]	2.1	74	67	116	312
MI, MS	69	3.1	2.1	1.8	1.2	40	55	155	380
MS	45	5.2	5 7	2.7	3.0	70	92	46	42
MS	48	[illegible]	3 9	1 7	[illegible]	34	72	153	123
ASD	16	4.3	3 8	[illegible]	2 5	61	61	70	40
ASD	62	3.3	3.3	1.8	1.8	57	46	46	49
ASD	54	3 9	4.3	2.4	2.6	53	88	41	34
VSD	23	4.3	3.6	2.7	[illegible]	54	69	48	48
ASD	44	5 7	4 8	3 6	3.0	68	57	70	58
ASD	43	4.1	4.2	2.8	[illegible]	54	75	72	68
ASD	17	5.6	5 7	3.5	3.5	62	68	57	47
ASD	42	3 4	3 4	[illegible]	2.2	47	65	1 973	2 375

started within _ min after the start of methoxamine injection and was usually most marked after 4–6 min. The fall in heart rate was less pronounced in patients with marked cardiac failure than in the normal controls (Table IV). The two patients in whom no fall in heart rate was seen were one patient with severe mitral insufficiency and stenosis, slight aortic insufficiency and congestive cardiac failure cardiac function groups III and IV and one patient with moderate mitral stenosis, pulmonary emphysema, cardiopulmonary function group II. The rise in the v peak pressure and the Δv/ΔA ratio was not related to the fall in heart rate.

In six patients a heart rate between 35 and 40 was seen after methoxamine. One of these patients complained of dizziness. In this case cardiac output was also recorded the cardiac index fell from 1.8 before to 1._ after methoxamine. The other patients had no symptoms due to the bradycardia. In twelve patients the lowest heart rate after methoxamine was between 40 and 50/min. Three patients had atrio-ventricular block with Wenckebach's periods, and one sino-atrial block, during the first minute after methoxamine injection. In all cases only a few cardiac cycles were blocked. All of these patients had a good cardiac function, three had no symptoms and one moderate dyspnoea on exertion.

DISCUSSION

Stanfield and Yu (7) have suggested that the study of pulmonary capillary pressure record-

Table IV *Increase in systolic blood pressure and reduction in heart rate after injection of methoxamine 2 mg/min*

Patient group	No. of pats.	Increase in systolic BP (mmHg)	Heart rate, of rate before injection
Mitral insufficiency all cases	15	50	63
Mitral insufficiency pure	3	29	67
Mitral stenosis, all cases	21	59	67
Mitral stenosis, pure	7	66	74
Aortic insufficiency all cases	8	46	74
Aortic insufficiency pure	2	38	88
Atrial septal defect	11	45	68
Ventricular septal defect	1	36	70
Organic heart disease, funct. groups I and II	27	55	67
Organic heart disease, funct. groups III and IV	11	40	73
No organic heart disease	9	55	62
All cases	47	52	67

ing during methoxamine infusion may permit the diagnosis of mitral insufficiency without resource to left heart catheterization. We have found, like Braunwald et al. (2) and Stanfield and Yu (7), that the average $\Delta v/\Delta A$ is higher in a patient group with mitral insufficiency than in a group with mitral stenosis only but in contrast to these investigators we find a marked overlap between the different patient groups. In routine diagnostic work the most important question is whether a patient with mitral stenosis also has a mitral insufficiency In the solution of this problem, in the individual case, the methoxamine test during right cardiac catheterization is of very limited value.

As a result of a rise in systemic arterial pressure an increase in mitral regurgitant flow will be seen, according to the haemodynamic calculations by Gorlin et al. (4) and the experiments by Braunwald et al. (2). The height of the v-peak in the left atrial pressure wave, however will depend not only on the mitral regurgitant flow but also on the left atrial volume and on the distensibility of the left atrium and the pulmonary veins. In observations on different patients it is our experience that the height of the v-peak is an inaccurate indicator of the presence of mitral insufficiency In this experiment, however the same patient has been observed before and after the mitral regurgitant flow had presumably been increased by the methoxamine-induced pressor effect on the systemic arteries. Individual variations in the left atrium pressure/volume characteristics, therefore, will not influence the result of this examination. It is reasonable to believe that in this situation a closer relation between mitral regurgitant flow and the height of the -peak would exist. It must be admitted, however that in the case f excessively low or excessively high pressures the pressurevolume relations in the atria will be markedly changed (6). But the results seem to indicate that this relation is apparent only in cases with severe pure mitral insufficiency When a significant mitral stenosis is added, the expected rise in the v-peak is usually not seen. This may be explained if one considers that the mitral regurgitant flow will be proportional to the mitral regurgitant area. Patients with a combined mitral stenosis and insufficiency usually have stiff, calcified, non-distensible mitral valves, while in patients with a pure mitral insufficiency the mitral valves are often flexible. The insufficiency is sometimes (i.e. after a myocardial infarction) due to a poor papillary muscle function, and the mitral ostium may therefore be more dilated in these patients when the systemic pressure is rising, and thus the mitral regurgitation will be greater The apparently good correlation between $\Delta v/\Delta A$ ratio and the incidence of mitral insufficiency in Heikkilä's investigation in patients with recent myocardial infarction (5) can be explained by this mechanism.

As reported the bradycardia after methoxamine was less marked in patients with cardiac failure (5). The reason for this is probably the strong sympathetic drive in patients with cardiac insufficiency which counteracts the reflex bradycardia induced by methoxamine (1). Marked bradycardia and episodes of transient A–V block, as seen in four patients in this investigation, has been reported previously by Ueda et al. (8) The incidence of A–V block seems to be related to the injection rate. For therapeutic v use, therefore, methoxamine ought to be injected slowly under control of pulse rate or ECG

The cardiac output did not decrease after methoxamine, as reported in the literature (9) The effect of methoxamine on cardiac output and left-to-right shunts will be further investigated in this laboratory

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PRUSSIAN BLUE IN THERAPY OF THALLOTOXICOSIS

An Experimental and Clinical Investigation

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Abstract. In recent literature good results of Prussian Blue therapy in thallium poisoning in the rat have been described. In our experiments Prussian Blue in vitro exhibited far greater adsorptive capacity for thallium than activated charcoal. Prussian Blue therapy significantly lowered thallium concentration in the brain. A definite dose—residual thallium relationship was demonstrated in rats with thallium poisoning treated with Prussian Blue. Three patients with thallotoxicoses were treated with Prussian Blue and showed favorable clinical response. For the moment Prussian Blue seems to be the therapy of choice in thallotoxicosis.

Thallium poisoning has been a therapeutic problem ever since this intoxication has been encountered. Many antidotes have been tried and rejected. Lately the chelating agent sodium diethyl dithiocarbamate has been advocated (1, 3, 10, 13). We were able to show however that it causes a redistribution of thallium, leading to an increase in brain thallium concentration. Therefore we consider the use of this antidote to be contraindicated in thallium poisoning (5, 6, 11). Alternative therapies have been disappointing. Potassium salts promote urinary thallium excretion, but only to a limited extent (8). Activated charcoal given orally adsorbs thallium ions secreted into the intestine and thus increases fecal thallium excretion. This increase, however, is not substantial (8).

Prussian Blue, potassium ferric hexacyanoferrate(II) an inorganic pigment orally administered, has been used to accelerate the elimination of radioactive cesium from the body (9). The mechanism of this effect appears to be the exchange of potassium ions by cesium ions. Heydlauf (4) applied Prussian Blue in experimental thallotoxicosis in rats. It offered considerable protection by binding thallium ions secreted into the gut, increasing fecal thallium excretion. In his experiments, however, he paid no attention to cerebral thallium concentrations.

In our experiments we first compared the adsorption of thallium to Prussian Blue and to activated charcoal. Then we studied the influence of Prussian Blue therapy on cerebral thallium levels in the rat. Finally we investigated the effect of Prussian Blue in three patients with thallotoxicosis.

MATERIAL AND METHODS

Materials

Prussian Blue was obtained from British Drug Houses. (Recently they informed our supplier that Prussian Blue was no longer in supply. It is now supplied by Chroma Gesellschaft, Stuttgart-Untertürckheim, Germany.) For adsorption experiments a small amount of Prussian Blue was made by adding stoichometric amounts of ferric chloride to potassium hexacyanoferrate(II) or ferrous sulfate to potassium hexacyanoferrate(III). The precipitates were washed by centrifugation and dried in vacuo. The activated charcoal used was Norit B powder.

Analyses

Thallium was determined according to Wolf and Lenstra (15).

Adsorption experiments

Suspensions of 100 mg activated charcoal or five preparations of Prussian Blue in 5 ml 0.15 M sodium nitrate were stirred and dialyzed at 37°C against 20 ml thallium nitrate solutions of varying concentrations in 0.15 M sodium nitrate. After 8 h the thallium concentration in the outer compartment was determined.

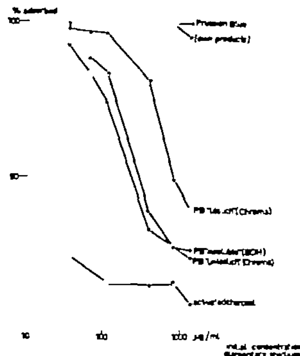


Fig 1 The adsorption of thallium to activated charcoal and various Prussian Blue preparations.

Animal experiments

T 35 female Wistar-rats, divided into seven groups of five animals, 0.075 mM kg thallium nitrate in glucose 5% solution was administered by intraperitoneal injection. After 24 h one group was killed. Three of the remaining groups were subsequently treated with 50 mg Prussian Blue suspended in saline by gavage twice daily. The other

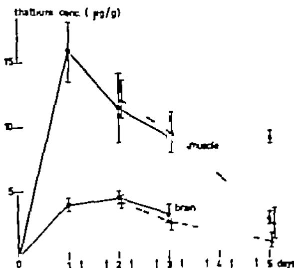


Fig Influence of the oral administration of Prussian Blue on thallium levels in the rat. ↑ Prussian Blue, 50 mg orally —●— control group ▲ treated group I, S.D (=5).

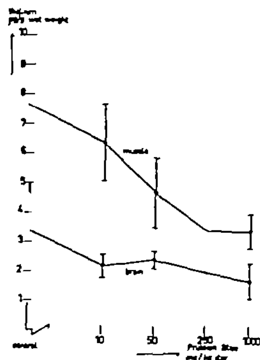


Fig 3 Dependence of tissue thallium concentration (± S.D.) on dose of Prussian Blue.

three groups served as controls. At 48, 72 and 96 h after the thallium administration one control and one treated group were killed. Thallium was determined in the brain and in muscle specimen (quadriceps).

T five groups of five female Wistar-rats 0.1 mM/kg thallium nitrate was administered intraperitoneally. After 24 h four groups were treated by gavage once daily with 10, 50, 250, or 1 000 mg kg Prussian Blue, respectively suspended in 15% mannitol to prevent obstipation. The control group received 15% mannitol only. After four days of treatment the animals were killed and thallium was determined in the brain and in muscle specimen (quadriceps).

RESULTS

In the in vitro experiment Prussian Blue exhibited a far greater adsorptive capacity for thallium ions than activated charcoal (*Fig. 1*)

After four days of Prussian Blue therapy the thallium concentration in the brain of the treated group was less than half this value in the control group. The muscle thallium concentration in the treated group was almost one fourth of this value in the control group (Fig. 2) These results, which show that treatment with Prussian Blue definitely lowers the thallium body burden, are in accord with the experiments of Heydlauf (4).

There is a definite dose—residual thallium rela-

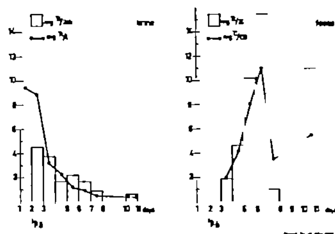


Fig. 4 Excretion pattern of thallium in patient treated with Prussian Blue.

tionship, which shows up clearly in the dose dependence of the thallium concentration in muscle after four days of Prussian Blue treatment. The dose dependence of the brain concentration is less clearcut (Fig. 3).

Clinical investigation

Having assessed that the findings of Heydlauf could be extended to brain thallium concentrations, we treated three patients suffering from thallium poisoning with Prussian Blue. The estimated ingestions of thallium sulfate were 400 mg in two patients, and 2 000 mg in the third. In this latter patient, who was admitted 14 days after the ingestion of thallium, a severe poisoning was observed with bloody diarrhea, followed by obstipation, paresthesias and severe pains in both legs, paresis, nystagmus, psychotic symptoms, cutaneous nodi on hands and feet, alopecia, hypertension and tachycardia. The glucose tolerance test was of the diabetic type serum transaminase levels were raised (GOT and GPT) as a sign of hepatic damage. Prussian Blue was given by duodenal tube in dose of 10 g twice daily The administration was continued for ten to fourteen days. By then urinary thallium concentration was less than 0.5 mg/L

Daily fecal and urinary thallium excretions were determined in all patients. Like most patients with thallotoxicosis these patients were severely obstipated. Therefore it was not possible in two of them to obtain feces every day not even when considerable amounts of mannitol solution were given as laxative. The excretion pattern of one of the patients who ingested 400 mg is depicted in Fig. 4 Here treatment was instituted one day after thallium ingestion. All patients recovered. The patient who had taken an otherwise lethal dose of thallium showed some residual neurological disturbances, namely a slight ataxia and paresis of the legs.

No side effects of Prussian Blue administration were observed.

DISCUSSION

In vitro thallium is much more strongly bound to Prussian Blue than to activated charcoal. This is probably due to exchange of potassium ions at the surface of the crystal lattice (7 14) by thallium ions (ionic radii 1.33 and 1.44 A respectively). The capacity to adsorb thallium ions is partly dependent on the method of preparation of the Prussian Blue. The so-called soluble Prussian Blue (12) contains substantially more potassium than the insoluble product. Having this property it should adsorb thallium ions more effectively and indeed it was reported to be a more effective antidote in thallium poisoning in the rat (2).

In vivo thallium ions secreted into the gut are bound to particles of Prussian Blue—which itself is not absorbed. This will prevent the reabsorption of thallium. As more and more thallium becomes bound in the gut and is excreted with the feces, the plasma level will decline. As a consequence urinary thallium excretion will diminish more rapidly than without Prussian Blue administration. This relative decrease, however is amply made up for by the increase in fecal excretion.

In the dose dependence experiment no saturation effects were observed. Theoretically it is possible that higher doses would be still more effective.

Our patients with thallium poisoning responded favorably to Prussian Blue therapy. Administration by duodenal tube was necessary because of pyloric spasm and gastric dilatation, which are often manifested in thallotoxicosis. Only when an effective laxative treatment is given can the therapy be successful.

As Prussian Blue is not absorbed in the gut and is stable in the conditions prevailing in the intestine no toxicity is to be expected. As there is no other effective therapy for thallium poisoning, and prolonged administration does not occur the risk of side-effects caused by contaminants is low and not in proportion to the benefit of this therapy.

For the moment Prussian Blue seems to be the antidote of choice in the treatment of thallium poisoning.

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FIBRINOLYTIC ACTIVITY IN HEMIPLEGIC PATIENTS

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Abstract The fibrinolytic activity after venous occlusion of the arms and the legs for 20 min has been studied in 19 patients with hemiplegia. The patients were divided into three groups according to degree of mobilization. In normals the fibrinolytic activity induced by venous occlusion is markedly higher (4 to 6 times) in the arms than in the legs. A marked fibrinolytic activity developed after venous occlusion of the arms in all but one of the patients. In contrast with normals, the patients confined to bed had almost the same fibrinolytic activity in the legs as in the arms, while in patients who were partly mobilized the activity of the legs, compared with that of the arms, decreased with increasing degree of mobilization. No difference was found between paralysed and non-paralysed arms and legs. The lower local fibrinolytic activity after venous occlusion of the legs, compared with that of the arms in normals, may therefore be due to emptying of the activator from the vessel wall, as a consequence of the habitually vertical position of human beings, a position favouring venous stasis.

That venous occlusion of the arms produces a local increase of the fibrinolytic activity of the blood was first reported by Clarke et al. (2) in 1960. According to Holemans (4) and Chakrabarti et al. (1), venous stasis enhances the fibrinolytic activity of the blood by liberating plasminogen activator from the walls of blood vessels. This theory has been recently substantiated by the works of Pandolfi et al. (10), who established a direct relationship between the degree of fibrinolytic response to venous stasis of a vessel and its content of plasminogen activator. Pandolfi et al. (11) and Nilsson and Robertson (9) found that stasis of the arms was followed by a much higher local fibrinolytic activity of the blood than stasis of the legs. This difference was reflected by a two- to fourfold higher concentration of plasminogen activator in the vessels of the arms than in those of the legs (9, 11).

The cause of this difference between the arms and legs is obscure. As known, however, at least during the major part of the day the veins of the legs have to withstand a much higher hydrostatic pressure than those of the arms. Furthermore walking, for example, calls for much more muscular work than ordinary use of the arms. To check whether these functional differences between arms and legs might help to explain why the fibrinolytic response of the arm is stronger than that of the leg, we investigated the fibrinolytic response to venous stasis of hemiplegic limbs of patients who had been confined to bed for various periods.

MATERIAL

Nineteen patients, 4 males and 15 females, aged 44–89 were studied. Seventeen had had hemiplegia, one had had quadriplegia following a traffic accident, and one had had eclampsia before parturition. To facilitate interpretation of the findings the patients were divided into three groups (Table I).

Group I

This group consisted of six paralysed and immobile patients. Five of them had had hemiplegia, since when they had been bedridden. They were not able to move by themselves, and were only turned in their beds from one side to another by nurses. The sixth patient (case 6) had had eclampsia before parturition 8 years previously and has since then been decerebrate.

Group II

To this group were assigned eight partially ambulant patients who had had hemiplegia. After having been bedridden for 10 days to 4 months, they had begun to recover and during the last week to 2 years they could sit in an armchair for 3–6 h and walk for 10–20 min

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Table I. *Survey of clinical material*

Pat. no.	Age	Sex	Hemiplegic for	Ambulant for
Group I				
1	76	♀	2 y	0 d
2	89	♀	2 y	0 d.
3	76	♀	1 y	0 d.
4	46	♀	15 d.	0 d.
5	78	♀	3½ y	0 d.
6	33	♀	8 y	0 d.
Group II				
7	64	♂	2 y	1½ y 3–6 h/d.
8	74	♂	1 y	6 mo 3–6 h/d.
9	79	♀	3 y	2½ y 3–6 h/d.
10	79	♀	6 y	4 y 3–6 h/d.
11	53	♀	25 d.	1 week, 3–6 h/d.
12	47		17 d.	1 week, 3–6 h/d.
13	72	♀	1½ y	10 mo 3–6 h/d.
14	41	♀	1½ y	6 mo., 3–6 h/d.
Group III				
15	69	♀	5 mo	4½ mo., 10–14 h/d.
16	31	♂	2 weeks	1½ y 10–14 h/d.
17	54	♀	1 y	10 mo., 10–14 h/d.
18	64	♂	5 mo.	2 mo 10–14 h/d.
19	71	♀	1½ y	5 mo., 10–14 h/d.

day I other words, they spent 3–6 h day in a more or less upright position.

Group III

This group comprised only ambulant patients. All except one had also had hemiplegia. They had first been confined to bed for some time, but had then been mobilized and rehabilitated. By the time of examination they had for weeks or months spent 10–14 h day out of bed, mostly sitting in an armchair but also walking for few minutes at a time, i.e. they had been in a more or less upright position for as long time a day as most ordinary persons. One patient (case 16), had had quadriplegia following an accident. He was first confined to bed for about 6 months, after which he began to recover and during the last 18 months he got about on crutches.

Normal controls

Since the patients were on the average above 67 years, and since most of them were women, we selected healthy women over 67 years as controls concerning the ffect of venous occlusion of the arms and legs on the fibrinolytic activity These women were part of the material used by Robertson and Nilsson (13) in an investigation of the effect of venous occlusion on the fibrinolytic activity in large series of normals of different ages.

METHODS

Venous occlusion was produced by placing a sphygmomanometer cuff around the upper arm and thigh, inflating it to pressure midway between the systolic and diastolic blood pressure for 20 min. Blood samples were taken before the cuff was inflated and at the end of 20 min, immediately before it was deflated, from an antecubital vein and from the distal part of the long saphenous vein, respectively The interval between venous occlusion of the paralysed and the non-paralysed limb was 15–30 min.

Fibrinolytic studies

The following determinations were made: fibrinolytic activity of resuspended euglobulin precipitate on unheated bovine fibrin plates, plasminogen, inhibitors of plasminogen activation by urokinase and α_2-macroglobulin. The methods used have been described lsewhere (3, 7 12). Fibrinogen was determined according to Nilsson and Olow

Table II. *Mean values of the fibrinolytic components before venous occlusion of the arm*

p = significance in values between patients and normals. N.S. = non-significant

	Normal	Patients Group I	*p*	Group II	*p*	Group III	*p*
Fibrinolytic activity on unheated fibrin plates (mm²)							
Mean	29	79	<0.05	43.9	N.S.	24	N.S.
S.D	30.7	23.8		30 8		33.8	
Fibrinogen (g/100 ml)							
Mean	0.30	0.43	N.S.	0.49	<0.01	0.36	N.S.
S.D	0.05	0.15		0.08		0.08	
α_2-macroglobulin (%)							
Mean	122	103	N.S.	139	N.S.	125	N.S.
S.D.	20	19 6		39		41	
Urokinase inhibitors (%)							
Mean	115	139	N.S.	160	<0.05	114	N.S.
S.D	20	37		62.3		33	

Table III. *Mean values of fibrinolytic activity after venous occlusion of the arm and leg (resuspended euglobulin precipitate on unheated fibrin plates, mm²)*

p = significance in values between patients and normals. N.S. = non-significant

	Non-paralysed limbs		Paralysed limbs		Significance between values for paralysed and non-paralysed limbs	
	Arm	Leg	Arm	Leg	Arm	Leg
Normal						
Mean	372	74	372	74	—	—
S.D	102	57	102	57		
Group I						
Mean	348	301	333	201	<0.05	<0.01
Range	305–465	221–383	248–412	192–231		
p	N.S.	<0.001	N.S.	<0.001		
Group II						
Mean	402	148	322	164	N.S.	N.S.
Range	251–515	26–230	136–455	100–253		
p	N.S.	<0.001	N.S.	<0.001		
Group III						
Mean	307	68.4	216	33	N.S.	N.S.
Range	74–466	0–159	69–318	0–89		
p	N.S.	N.S.	N.S.	N.S.		

(II). Only fibrinogen values for blood collected with EACA are given.

All these determinations were made before venous occlusion. In samples collected immediately before the end of venous occlusion, only the fibrinolytic activity of resuspended euglobulin precipitate on fibrin plates was assayed.

RESULTS

The mean levels of fibrinogen and urokinase inhibitors determined in the arm veins before venous occlusion were found to be somewhat higher in the patients than in the controls (Table II). It was especially one patient in group II who had unusually increased levels of these components. The α_2-macroglobulin level and the plasminogen level were within normal limits.

The mean and the individual levels of fibrinolytic activity after venous occlusion of the arms and the legs are given in Table III and Fig. 1

A marked fibrinolytic activity measured on unheated fibrin plates was found after venous occlusion of paralysed and non-paralysed arms of all the patients except one. In the second and third groups of patients no difference in fibrinolytic response to venous occlusion was demonstrable between paralysed and non-paralysed limbs. But in the first group of patients the fibrinolytic activity that developed during venous stasis in the non-paralysed limbs was somewhat higher than that in paralysed limbs (Table III).

But the groups differed in their fibrinolytic response of the leg veins to venous occlusion. In the first group, where the patients were still bedridden, venous occlusion of the leg produced an abnormally high local fibrinolytic activity. In fact the response of the leg veins was almost as strong as that of the arm veins.

In the second group, where the patients were in upright position for 3–6 h day enous occlusion of the leg also produced a marked fibrinolytic activity. The response was less strong than in the first group, but was significantly stronger than normal.

In the third group, where the patients had been out of bed for 10–14 h a day the post-occlush increase of the local fibrinolytic activity of the leg veins was markedly smaller than in the other two groups and did not differ from that in the controls.

DISCUSSION

It has been generally believed that the increase of fibrinolytic activity developing during venous oc

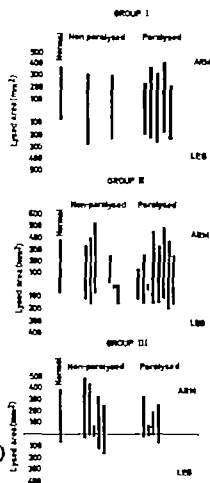


Fig 1 The individual fibrinolytic activity before and after venous occlusion of the arm and leg in patients (resuspended euglobulin precipitate on unheated fibrin plates, mm²). The difference between the values obtained before (Table II) and at the end of occlusion (Table III) of the arms of the patients was significant ($p < 0.001$).

clusion is due to the release of plasminogen activators from the vessel walls into the blood stream (1 4 9). It has been shown in normals that the fibrinolytic response of the arm veins to venous occlusion is much stronger than that of the leg veins (9 11), but no satisfactory explanation for this difference has been available.

In this study we found a relationship between the erect position of the patients and the difference between the *fibrinolytic response of* the upper and lower limbs to venous occlusion.

In patients who had been bedridden for more than 15 days, venous occlusion induced a marked fibrinolytic response in both paralysed and non-paralysed legs. A moderately marked fibrinolytic response was also obtained in both legs in patients who had been out of bed for only 3–6 h a day. The response was less marked than in the bedridden patients, but it was still abnormally strong, compared with the controls. On the other hand the fibrinolytic response of the leg veins of the patients who had been out of bed for as many hours a day as most healthy persons was normal. The fibrinolytic response of the arm veins to venous occlusion was the same in each group. These findings strongly suggest that the erect position of man might at least partly explain why the fibrinolytic response of the leg veins to venous occlusion is not so strong as that of the arm veins.

It is evident that the motor function of the limbs cannot explain the difference between the fibrinolytic response of the upper and lower limbs, for the fibrinolytic response was the same whether the limb was paralysed or not.

On the other hand, no correlation was found between the local fibrinolytic activity of the limbs and the physical activity of the patients. The physical activity of the patients was limited. The patients spent most of their time in an armchair and some of them could walk for a few minutes a day and a few were relatively active. The fibrinolytic response of the legs of these patients did not vary with their physical activity but with the period of time the patients were out of bed and in upright position.

In the first group the local fibrinolytic response of non-paralysed limbs was found to be only a little higher than that of paralysed limbs. We are not sure whether the motor functions of the extremities play any role in the development of the local fibrinolytic response or whether the difference was due to chance, since this group was very small.

Judging from our findings, the mechanism of the lower local fibrinolytic response of the leg veins to venous occlusion can be explained as follows. Because of the upright position of the human body during the daytime, the veins of the leg have to withstand a much higher hydrostatic pressure than the veins of the arm, i.e. there is a little but continuous stasis in the leg veins. This physiological stasis stimulates a continuous liberation of plasminogen activators from the vessel walls. This continuous release prevents an accumulation of plasminogen activators in the walls. The leg veins are therefore not able to respond

to venous occlusion, owing to the lack of plasminogen activators in the vessel walls.

If our postulation is correct, the fibrinolytic response of the arm veins of a patient with venous stasis of the upper limbs should be very little, as in the leg veins. In fact, a patient with venous stasis of the upper limbs owing to pulmonary fibrosis and total heart failure was studied and it was observed that the local fibrinolytic response of the arm veins to venous occlusion was barely discernible. This is the only case studied so far.

We think that this investigation may also allow some considerations on the development of thrombosis in the legs in the postoperative period and in patients confined to bed for a long time. When thrombotic complications occur in the postoperative period, they usually do so within 10 days of the operation. In patients who have been in bed continuously for more than 15 days after operation or because of some other condition thrombosis is rather rare. In the present material no thrombotic accident had been noticed. When a patient has been in bed for about 15 days the content of plasminogen activators in the vessel walls is increased, as shown by a high fibrinolytic response to venous occlusion. This may help to prevent the development of thrombosis in the legs.

In conclusion, it seems that the weaker fibrinolytic response of the leg veins to venous occlusion is closely related to the erect position of the human body since a marked fibrinolytic activity developed in the leg veins of patients who had been bedridden for more than 15 days.

ACKNOWLEDGEMENT

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Frequency variations in the ECCG An increase of the frequency will shorten the R-R intervals, and the strings of the QRS contours are directed from above downwards to the left (Fig. 3) The screen may be provided with a grid indicating R R intervals of some calibrated heart rate values in order to facilitate heart rate readings (Fig. 4)

S-T and T changes in the ECCG S-T and T changes were usually quite apparent. In Fig. 4 the contourogram shows a distinct S-T elevation and widening of the Q wave, both being signs of an anterior wall myocardial infarction. The upper part of the picture also shows frequent muscle potentials. In the lower part of the ECCG the patient had been assisted in finding a more comfortable position in his bed. S-T depression was better displayed with the IMF turned off In Fig. 5 the characteristic S-T changes induced by digitalis are seen. With IMF on, this portion of the ECCG would occur as a dark field.

Premature beats in the ECCG Ventricular premature beats (VPB) were recognized in the ECCG pattern even if they appeared in the ordinary QRS strings, since widening of the QRS complex and changes of the repolarisation were evident. In some cases a compensatory pause was obvious. By using the IMF the compensatory pause was more easily detected (IMF turned on) (Fig. 6)

Bigeminy and similar types of irregularities gave a characteristic pattern to the ECCG but were sometimes difficult to interpret (Figs. 7 and 8).

Atrial fibrillation. This disturbance resulted in a typical ECCG pattern regardless of whether the intensity modulation function was used or not (Fig. 9) In the left part of the contourogram the range of R R variation is obvious and an estimation of the median of the R R time interval is possible.

Atrial flutter The sawtoothed baseline of this type of arrhythmia was usually seen. The QRS contour appeared more or less irregular depending on the degree of variation in A V blocking, which gave the contourogram a typical appearance and facilitated this diagnosis (Fig. 10)

The contourogram in A V block The P wave was often distinct in the ECCG A V block of different degrees and types was therefore easily recognized.

Wenchebach type of A V-block With blocking, the QRS strings appeared in groups of two or more. The diagnostically important prolongation of the P-Q time interval was often obvious (Fig. 11)

Complete (third degree) heart block The P waves were generally seen in diagonal lines or occurred in relatively regular zig zag patterns (Fig. 12).

Tachyarrhythmias. In Fig. 13 the transformation of atrial fibrillation with VPB into ventricular tachycardia is shown. Fig. 14 (with IMF on and with continuous erasing) shows for comparison the pattern of sinus tachycardia.

DISCUSSION

The clinical testing of the ECG contourograph, which has been in current use in our clinic for the last two years, has shown that the principle of contourography is valuable for the ECG monitoring of critically ill patients. Also it provides means for the diagnosis of arrhythmias and other heart disturbances.

In order to determine whether the ECCG is normal or not, the trained observer needs just a quick glance at the screen. Differential diagnosis of arrhythmias is also easy to learn since many types of arrhythmias exhibit characteristic ECCG patterns. When cardiac rhythm is extremely regu-

Fig 7 Male, 70 years. Clinical diagnosis, myocardial infarction (second day). Upper two-thirds SA rhythm with sparse VPB. Lower third trigeminy No compensatory pause after the premature beat, not disturbing frequency notable. Sweep 25 mm/s. 50 lines. Intensity modulation.

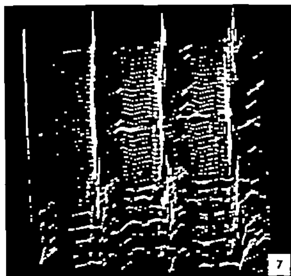
Fig 8 Male 70 years. Clinical diagnosis, barbiturate and digitalis poisoning. Nodal rhythm. Frequent ventricular premature beats in upper half, in lower half bigeminy Sweep 50 mm/s. 100 lines. Intensity modulation.

Fig 9 Male 76 years. Clinical diagnosis: myocardial infarction (fourth day). Atrial fibrillation with ventricular premature beats of rS-configuration. Sweep 25 mm/s. 50 lines.

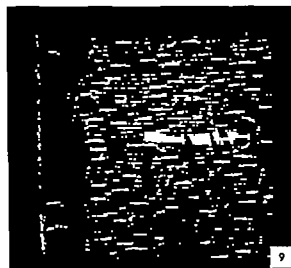
Fig 10 Male, 67 years. Clinical diagnosis, digitalis over dosage. In the ECCG the uneven distribution of the QRS complexes speaks against complete heart block or nodal bradycardia. Oesophageal lead showed flutter Sweep 25 mm/s. 50 lines.

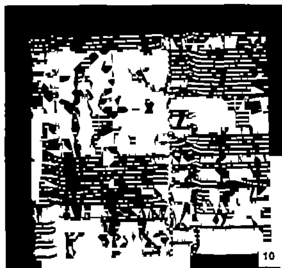
Fig 11 Male, 60 years Clinical diagnosis, myocardial infarction (second day). Wenchebach type of second degree heart block. Commonly blocking after every third P-wave. Sweep 25 mm/s. 50 lines.

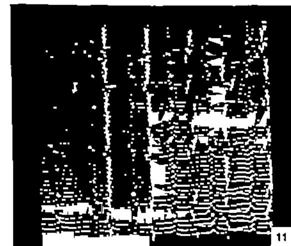
Fig 12 Male, 69 years. Clinical diagnosis, myocardial insufficiency Complete heart block. P waves make wave-like contourlines. Sweep 25 mm/s. 50 lines.


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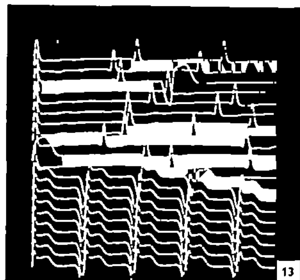


Fig 13 Male, 66 years. Clinical diagnosis: pulmonary oedema. The upper half of the tracing shows atrial fibrillation with VPB, the lower half ventricular tachycardia (95 beats min). Sweep 25 mm s, 20 lines

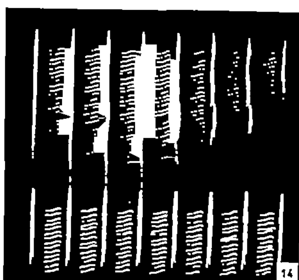


Fig 14 Male 17 years. Clinical diagnosis: diabetic coma. Sinus tachycardia. Sweep 25 mm/s. 50 lines. Intensity modulation.

lar as in third degree heart block and idioventricular rhythm, contourography may be a definite diagnostic aid. Supraventricular tachycardias with left bundle branch block may possibly be differentiated from a ventricular tachycardia, which is slightly irregular. Periodic irregularities, like Wenchebach block, are easily detected with the ECCG. When the ventricular rate in complete heart block is an even multiple of the atrial rate, this arrhythmia may be mistaken for second degree heart block. With contourography this risk diminishes since longer periods of time can be scrutinized very quickly.

The contourograph offers a means to study the effect of drugs on the heart rhythm. For instance, in atrial fibrillation with rapid ventricular frequency the scatter of the R-R time intervals during treatment with digitalis is changed in a way which is observable in the contourogram.

Rapid reproduction and analysis of ECG data collected over long periods is sometimes needed, e.g. in patients under investigation for suspected episodic arrhythmias. The contourograph may here be used to visualize the tape-recorded ECG data with the tape recorder speeded up several times to shorten the time for off-line screening. Another way is to store the contourograms with the aid of a video tape recorder. As interpretation of one ECCG only requires a few seconds, pictures taken during 4 hours would probably not require more than 10 minutes observation time. The contourograph display imposes a high luminescence and may be transmitted by internal TV for educational purposes or remote supervision.

ACKNOWLEDGEMENTS

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MILD CARBOHYDRATE INTOLERANCE DEVELOPING INTO CLASSIC JUVENILE DIABETES

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Abstract. Mild carbohydrate intolerance in children and young people is very seldom seen and the natural history of the disease is unknown. The work is study of the plasma insulin response to glucose and tolbutamide in young men during state of mild carbohydrate intolerance as well as after changing into severe carbohydrate intolerance two and half years later. A significant but delayed plasma insulin response was seen during the state of mild carbohydrate intolerance after oral and intravenous glucose, while no response occurred after intravenous tolbutamide. No plasma insulin response at all to either glucose or tolbutamide was found during the state of severe carbohydrate intolerance.

It is an old clinical experience that spontaneous diabetes in man appears in two different types: juvenile diabetes and maturity-onset diabetes. Juvenile diabetes sets in acutely with severe symptoms and is characterized by carbohydrate intolerance with a pronounced tendency to ketoacidosis, and insulin treatment is obligatory. Maturity-onset diabetes develops gradually and the carbohydrate intolerance is mild. Symptoms are few there is no ketosis and there is seldom need for insulin treatment.

Diabetes appearing in middle-aged and elderly people is usually of the maturity-onset type, but severe "juvenile type" develops in 10 to 20%. Children and young persons develop severe diabetes of juvenile type, and it was previously supposed that mild diabetes of maturity-onset type did not occur or was exceedingly rare in childhood and youth.

During a study of the plasma insulin response to glucose and tolbutamide in a large and unselected group of diabetic patients we have come across 15 young subjects exhibiting the metabolic feature and the plasma insulin responses of maturity-onset diabetes. None of the patients were obese. The glucose tolerance test curves were diabetic, and there was glucosuria but never ketonuria. The diurnal blood glucose level was elevated, but some of the subjects had normal fasting blood sugar. The plasma insulin response pattern of young patients with mild carbohydrate intolerance has been described in preliminary reports from this laboratory (6, 7).

We have previously stated that the natural history of mild carbohydrate intolerance in children and young people is unknown, and we proposed the possibilities that the disorder may represent either an early phase of classic juvenile diabetes or perhaps maturity-onset diabetes appearing at an exceptionally early age.

One of our patients has now developed classic juvenile diabetes. This case is reported because of the important implications for the understanding of the evolution of mild carbohydrate intolerance in young people.

METHODS

Four tests were used to describe the insulin response pattern: an oral glucose tolerance test with 100 g of glucose, a double oral glucose tolerance test with 100 g of glucose given at one hour interval, an intravenous glucose tolerance test, and a tolbutamide test. Blood glucose was determined by an enzymatic method (3). Plasma insulin was measured by Hales and Randle double antibody technique (5) after addition of EDTA. Both estimations were performed on venous blood.

CASE REPORT

The patient was 29-year-old army officer with no family history of diabetes. He was admitted for the first

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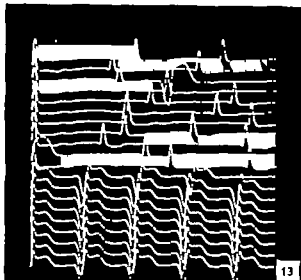


Fig 13 Male, 66 years. Clinical diagnosis, pulmonary oedema. The upper half of the tracing shows atrial fibrillation with VPB, the lower half ventricular tachycardia (95 beats/min). Sweep 25 mm/s, 20 lines.

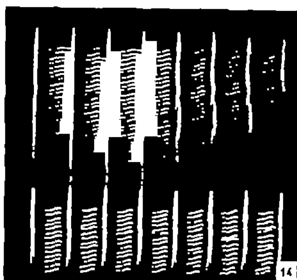


Fig. 14 Male 17 years. Clinical diagnosis, diabetic coma. Sinus tachycardia. Sweep 25 mm/s, 50 lines. Intensity modulation.

lar as in third degree heart block and idioventricular rhythm, contourography may be a definite diagnostic aid. Supraventricular tachycardias with left bundle branch block may possibly be differentiated from a ventricular tachycardia, which is slightly irregular. Periodic irregularities, like Wenckebach block, are easily detected with the ECCG. When the ventricular rate in complete heart block is an even multiple of the atrial rate, this arrhythmia may be mistaken for second-degree heart block. With contourography this risk diminishes since longer periods of time can be scrutinized very quickly.

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diabetes with symptoms. The children were siblings of children with diabetes mellitus. Kosaka et al. (8) followed the plasma insulin response to glucose during exacerbations and remissions of the disease in a 17-year-old girl with mild diabetes. They found that the blood sugar and plasma insulin changed inversely with each other.

These reports seem to indicate that young patients with mild diabetes sometimes get a normal carbohydrate tolerance during treatment, while some of the patients develop classic juvenile diabetes.

Our case is the first reported in which the role of the endogenous plasma insulin level has been elucidated both during the state of mild carbohydrate intolerance and during the development of classic juvenile diabetes.

During the state of mild carbohydrate intolerance a significant but delayed plasma insulin response was found after oral and intravenous glucose, while no response occurred after intravenous tolbutamide. During the state of severe carbohydrate intolerance no plasma insulin response at all was found either to glucose or tolbutamide.

It would be interesting to know how often this sequence of events takes place in the evolution of classic juvenile diabetes. An even more important question remains: should all young patients with asymptomatic mild diabetes be treated with diet and oral antidiabetic drugs to prevent the development of classic juvenile diabetes?

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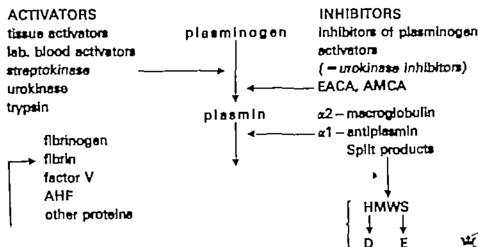
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Urinary tract haemorrhages may be caused by increased fibrinolytic activity Cyklokapron reduces or arrests fibrinolytic bleeding

In recent years fibrinolytic inhibitors have found widespread use in a number of haemorrhagic conditions, particularly in urinary tract haemorrhages and in connection with prostate surgery. Urine contains urokinase. This enzyme activates the conversion of the plasminogen present in the blood and blood clots into the proteolytic enzyme plasmin, which dissolves clots and thus sus*tains various types of haemorrhage in the urinary tract.* Cyklokapron produces a haemostatic effect by counteracting the activity of urokinase.

The Swedish investigators, Lennart Andersson and Inga Marie Nilsson, have obtained good clinical results by administering Cyklokapron to patients suffering from haemorrhages in the upper and lower urinary tract as well as postoperative bleeding following prostate surgery. Patients suffering from haematuria as a result of general fibrinolysis were also included in the investi*gation. Bleeding ceased completely in all the patients* in the latter group, as was the case with most of the other patients.

the fibrinolytic system



ANDERSSON, L & NILSSON, I M: AMCA (aminomethyl cyclohexane carboxylic acid, Cyklokapron ®) potent haemostatic agent in urinary tract bleeding. Scand J Urol Nephrol 3 ([illegible]) p [illegible]

Acta med. scand. Vol. 189 pp. 341–348 1971

RENAL TUBULAR DYSFUNCTION AND HYPERGAMMAGLOBULINAEMIA

Electrolyte Balance Electron Microscopic and Immu ohistochemical Studies

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Abstract. A report is given on female patient, 3? years of age, ith history of slight joint distress, recurrent dependent oedema, hypokalaemia, hypergammaglobulinaemia and positive tests for rheumatoid factor. Electrolyte balance studies revealed latent renal tubular acidosis (RTA), impaired renal pH-regulation and impaired renal conservation of sodium and potassium. Systemic pH was found to be largely dependent on the dietary load of sodium chloride. Correction of the hypokalaemia resulted in frank systemic acidosis, whereas persistent hypokalaemia was associated with alkalosis. The situation thus differed from that found in primary RTA. Electron microscopic and light microscopic studies from renal biopsy revealed simultaneous signs of tubular and interstitial nephritis and slight glomerular reaction. Immunoglobulin deposits were seen in the tubuli and the glomerular basement membrane by the immunofluorescence method.

Different kinds and degrees of renal tubular dysfunction, including renal tubular acidosis (RTA), diabetes insipidus, hypokalaemia, glucosuria and aminoaciduria, are known to occur in hyperglobulinaemic states (6 11). In particular RTA has been sought extensively and found in association with connective tissue diseases, Sjögren s syndrome, hypergammglobulinaemic purpura, idiopathic hypergammaglobulinaemia, chronic active hepatitis, cryoglobulinaemia, multiple myeloma, sarcoidosis and Hodgkin s disease (6).

We studied a patient with a history of slight joint distress, hypergammaglobulinaemia, positive tests for rheumatoid factor and recurrent dependent oedema. Electrolyte balance studies revealed tubular dysfunction, including latent RTA, impaired renal pH regulation, and impaired sodium and potassium conservation. A renal biopsy study by electron microscopy (EM) and immunofluorescence (IF) techniques revealed unusual histological findings and the presence of gammaglobulin.

CASE REPORT

The patient was a 32-year-old woman. She had suffered from periods of dependent oedema since the age of 15 Episodes of severe muscular eakness had been induced by small doses of chlorthalidone. At the ge of .3 she had normal pregnancy and delivery At 27 she had arthralgia in the digital joints of both hands. Radiographs showed normal findings, but tests for rheumatoid factor were positive and she was given short course of treatment with chloroquine The pain later disappeared completely

One year prior to the balance studies reported (Fig. 1), the patient was admitted because of acute abdominal pain. A normal appendix was removed and serum potassium concentration of 2.2 mEq/l as detected. The patient had moderate dependent oedema. The blood pressure was 115/90 mmHg. There were no signs of orthostatic hypotension or venous or cardiac insufficiency Daily renal excretion of 17-keto and 17-ketogenic steroids as normal. Serum gammaglobulins were increased, 1.85 g/100 ml, and immunoelectrophoretic analysis revealed slight increase of the IgG and IgM fractions. Serum albumin as normal, 4.1 g/100 ml. The Waaler-Rose test for rheumatoid factor was strongly positive (1 400). Tests for LE cells ere negative, but antinuclear antibodies giving speckled pattern of staining were detected by the IF method.

The endogenous creatinine clearance and urine concentration capacity were normal. Ammonium chloride loading, how ver failed to decrease the urine pH below 6.1 On normal diet and ithout diuretics the serum potassium increased to 4.1 mEq/l. When the patient was receiving .5 mg chlorthalidone and 50 mEq potassium citrate/day continuously she had several episodes of severe muscular eakness and the serum potassium as .1 .5 mEq/l. When 75 mg spironolactone was added

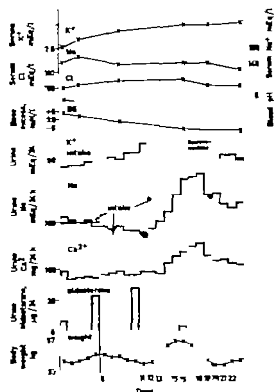


Fig. 1. Changes in body weight, blood chemistry, renal excretion of electrolytes and aldosterone under different dietary sodium loads. The diet was isocaloric and contained 80 mEq K /day. The diet high in sodium achieved by adding 250 mEq sodium chloride. Spironolactone, 300 mg/day given as indicated.

she improved and the serum potassium rose to 2.7–3.5 mEq/l. All medication was then stopped for 10 days, after which the patient was admitted for balance studies.

Balance studies

On admission the blood picture was normal. The urinary sediment was normal and there was no proteinuria. The ESR varied between 32 and 51 mm/h.

At the beginning of the balance studies, which are summarized in Fig. 1, the patient had hypokalaemic (2.6 mEq/l) non-respiratory alkalosis (pH 7.48, base excess +4.2 mM/l) with a normal serum chloride level (100 mEq/l). On a normal diet the potassium balance was positive and the serum potassium concentration increased. Faecal electrolytes were analysed on several occasions and found to be in the order of a few mEq of sodium and potassium per day. The patient never had diarrhoea. The faecal loss of these electrolytes was considered negligible and has been ignored in calculating the results, since they were not followed continuously. The patient lost some weight and the oedema subsided. With a low sodium diet, aldosterone excretion (9) increased and the potassium balance became negative. Hypokalaemia did not develop, however. Renal sodium conservation was inefficient and the patient lost some 300 mEq sodium in a week, which is more than normal under these conditions (3). 250 mEq of sodium chloride daily was then added to the diet. The sodium balance became positive, the weight increased, aldosterone excretion was suppressed and the potassium balance remained unchanged. A non-respiratory hyperchloraemic acidosis developed (pH 7.33, base excess 5 mM/l, serum chloride 110 mEq/l). Despite this the urine pH did not fall below 5.9 even during loading with ammonium chloride (100 mg/kg). Spironolactone increased the excretion of sodium but did not affect renal potassium excretion. Renal excretion of calcium was not increased but was correlated in a normal way with the excretion of sodium. Serum calcium and phosphate were normal. Tests for urine concentration and dilution were normal. The absence of liver disease was verified by a normal bromsulphonphthalein test and a normal morphological light microscopic structure in a percutaneous liver biopsy specimen. The patient was discharged and continued to attend the Outpatient Department. She did well when given 100 mg spironolactone and 13 mEq potassium citrate per day. Serum electrolytes were within normal limits throughout this period. Oedema was slight or absent. When the patient had been normokalaemic for 6 months the ammonium chloride loading test was repeated with the same result as before. After that a percutaneous renal biopsy of the left kidney was performed.

Renal biopsy study

The biopsy specimen was divided in chilled Ringer solution for light microscopic (LM), electron microscopic (EM) and immunofluorescence (IF) examination.

Light microscopy and electron microscopy

Tissue for LM and EM studies was fixed in phosphate-buffered 3% glutaraldehyde and postfixed with phosphate-buffered 1% osmium tetroxide before dehydration. Epon-embedded (5) pieces were sectioned at 0.08 μ and double stained with 1% uranium acetate in 50% ethanol and in lead citrate (8). Light microscopic sections, 1 μ thick, were stained with 1% methylene blue in 1% sodium borate.

The LM study revealed 11 fairly normal glomeruli. The number of epithelial cells seemed to be slightly increased and some mesangial areas appeared prominent. The basement membrane appeared normal. Numerous tubules with pycnotic nuclei seemed atrophic, collapsed, and often had starlike contours (*CT* in Fig. 2). The cell cytoplasm showed some vesicular vacuolation and was clearly thinner than normal. There were also a few dilated tubules with protein casts and a thickened basement membrane. In the tubular epithelium, cells resembling lymphocytes were found (Fig. 6). Between the tubules, lymphocytes, plasma cells, histiocytes and some mast cells were seen (*Fig. 4*). This reaction was also seen around some distal tubules, but distal tubular cells appeared normal. Many interstitial cells had dark and condensed nuclei and vesicular vacuolation in the cytoplasm, as well as green-staining granules, the size of which clearly varied. Aggregates of degenerated, possibly tubular cells were found around the glomerular hilus (Fig. 3). The size of the JGAs studied was normal.

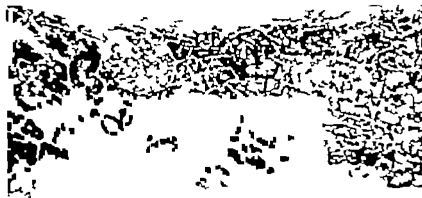


Fig. 2. Low power micrograph of the renal needle biopsy. T glomeruli with slightly increased cellularity. Under *CT* collapsed starlike tubuli found, under *VT* dilated normal looking tubuli, and above *DT* cells suggested to be destroyed tubular cells in the neighbourhood of the two glomeruli. Slight interstitial inflammatory reaction found between the tubuli. Epon-embedded section, methylene blue stain. 100.

The EM study of the glomerulus revealed an unevenly thickened basement membrane. Thickening was most prominent in the mesangial areas where, in addition, the membrane was folded and the lamina densa often appeared compressed into serpentine bends (Fig. 5). Epithelial cells filled the spaces between the capillaries without displaying any abnormal changes.

Three types of tubules were seen. Tubules with casts inside the epithelium appeared atrophic, and the cast was often in contact with the tubular basement membrane. All other changes ere found in the proximal tubules. Atrophic tubules ith starlike contours showed epithelial



Fig. 3. Large area of degenerated tubular cells in the immediate neighbourhood of glomerulus. *a*, arteriole. 350. Fig. 7 is an electron micrograph of correspond-ing area.



Fig. 4. Chronic inflammatory reaction around an obliquely sectioned distal tubulus in the cortex. Numerous lymphocytes and monocytoid cells. A granular cell, obviously mast cell is seen (right lower corner). Cells ith dark staining angular nuclei appear to be degenerated tubular cells. 700

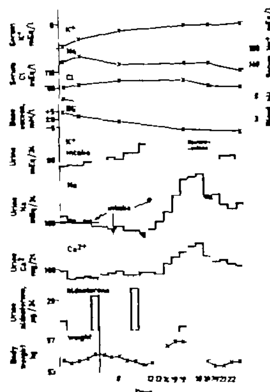


Fig. 1. Changes in body weight, blood chemistry, renal excretion of electrolytes and aldosterone under different dietary sodium loads. The diet was isocaloric and contained 80 mEq K /day. The diet high in sodium achieved by adding 250 mEq sodium chloride. Spironolactone 300 mg/day given as indicated.

she improved and the serum potassium rose to 2.7–3.5 mEq/l. All medication was then stopped for 10 days, after which the patient was admitted for balance studies.

Balance studies

On admission the blood picture was normal. The urinary sediment was normal and there was no proteinuria. The ESR varied between 32 and 51 mm/h.

At the beginning of the balance studies, which are summarized in Fig. 1, the patient had hypokalaemic (2.6 mEq/l) non-respiratory alkalosis (pH 7.48, base excess +4.2 mM/l) with a normal serum chloride level (100 mEq/l). On a normal diet the potassium balance was positive and the *serum potassium concentration* increased. Faecal electrolytes were analysed on several occasions and found to be in the order of a few mEq of sodium and potassium per day. The patient never had diarrhoea. The faecal loss of these electrolytes was considered negligible and has been ignored in calculating the results, since they were not followed continuously. The patient lost some weight and the oedema subsided. With a low sodium diet, aldosterone excretion (9) increased and the potassium balance became negative. Hypokalaemia did not develop, however. Renal sodium conservation was inefficient and the patient lost some 300 mEq sodium in a week, which is more than normal under these conditions (3). 250 mEq of sodium chloride daily was then added to the diet. The sodium balance became positive, the weight increased, aldosterone excretion was suppressed and the potassium balance remained unchanged. A non-respiratory hyperchloraemic acidosis developed (pH 7.33, base excess 5 mM/l, serum chloride 110 mEq/l). Despite this the urine pH did not fall below 5.9 even during loading with ammonium chloride (100 mg/kg). Spironolactone increased the excretion of sodium but did not affect renal potassium excretion. Renal excretion of calcium was not increased but was correlated in a normal way with the excretion of sodium. Serum calcium and phosphate were normal. Tests for urine concentration and dilution were normal. The absence of liver disease was verified by a normal bromsulphophthalein test and a normal morphological light microscopic structure in a percutaneous liver biopsy specimen. The patient was discharged and continued to attend the Outpatient Department. She did well when given 100 mg spironolactone and 13 mEq potassium citrate per day. Serum electrolytes were within normal limits throughout this period. Oedema was slight or absent. When the patient had been normokalaemic for 6 months the ammonium chloride loading test was repeated with the same result as before. After that a percutaneous renal biopsy of the left kidney was performed.

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Fig. 6 Interstitial reaction between the tubuli and lymphocytes (*L*) between the tubular cells. *M* monocytoid cell, around which an interstitial cell with large cytoplasmic vacuoles (one with dark core). × 4 300. The LM inset (left upper corner) shows a lymphocyte in the tubular epithelium (arrow). × 400.

Rabbits were immunized with human IgG separated chromatographically and incorporated with Freund' complete adjuvant. The resulting antiserum was fractionated with $(NH_4)_2SO_4$ to yield gammaglobulin, which was labelled with fluorescein isothiocyanate as described by Holborow and Johnson (4). To reduce non-specific staining, the conjugate was absorbed with acetonized liver powder.

Antiserum to the human $beta_{1c}$ component of complement was made in rabbits by the technique of Stratton (13) and gammaglobulin conjugated as described above.

Tissue sections were cut at 6 μ in a cryostat at −20°C, transferred to glass slides and air-dried at room temperature. They were washed in phosphate-buffered saline, pH 7.2, for 1 hour in a shaking apparatus, and fixed in 95% ethanol for 10 min. The sections were incubated in a moist chamber with one drop of fluorescein-conjugated anti-human globulin for 30 min at room temperature, washed again for 1 hour and mounted in buffered glycerol, pH 7.4. The Leitz Orthoplan microscope fitted with an Osram HBO-200 ultraviolet lamp was used in reading the results (filters UG 1 and K 430, dark-field condensor). The specificity of positive reactions was verified by absorption of labelled antiserum with specific antigen and blocking of reactions with unconjugated antiserum.

Linear staining of the glomerular basement membrane for IgG was found (Fig. 8). It was relatively weak, with focal accentuation in the mesangial areas. In the proximal convolution the immunoglobulin was visible as brightly fluorescent granules within the cells and in the lumina (Fig. 9). The tubular basement membrane was only weakly stained, and faint speckled fluorescence of cell nuclei was observed (Fig. 9). No staining of glomeruli or tubules was seen when labelled anti-$beta_{1c}$ antiserum was used.

DISCUSSION

It is difficult to decide the aetiological diagnosis of this patient, but a systemic connective tissue disease is suggested by several of the clinical findings and laboratory data, viz. articular pain, hypergammaglobulinaemia, antinuclear antibodies and high level of rheumatoid factors.

The patient became severely hypokalaemic and moderately alkalotic during sodium depletion with small doses of diuretics. Dietary sodium restriction induced a markedly negative potassium balance. These effects were at least partly due to an exaggerated aldosterone-dependent response to sodium depletion, which explains the good therapeutic effect of spironolactone. Potassium excretion was probably further enhanced by an increase in the bicarbonate/chloride ratio in the glomerular filtrate during alkalosis (2). Sodium chloride corrected the hypokalaemia, but simultaneously a frank hyperchloraemic acidosis developed. Normokalaemia during acidosis was not

Fig 7 Electron micrograph of an area with degenerated cells in the immediate vicinity of a glomerulus. At *A* the wall of the afferent arteriole. The cells are suggested to be tubular cells with collagen fibre bundles between them. Note the cytosomal granules commonly found in tubular cells (*small arrows*). These cells also display granules with homogeneous gray core (*larger arrows*). × 3 700.



Fig 8 Linear deposition of immunoglobulin along the glomerular basement membrane. Kidney section stained with fluorescein-labelled anti-human IgG antiserum.

simply the result of an altered distribution of potassium since the potassium balance changed in a positive direction during acidosis.

The persistence of non-respiratory alkalosis during sodium depletion indicated that the maximal proximal tubular hydrogen secreting capacity responsible for the bulk of the bicarbonate reabsorption was not severely impaired. On the other hand, ammonium chloride loading revealed a constant inability to decrease urine pH during acidosis. This implies a deficiency of the distal,

gradient-forming hydrogen ion secreting mechanism. The degree of acidosis resulting from this defect was largely dependent on the dietary sodium chloride load and the aldosterone secretion rate. The proximal tubular sodium reabsorption is assumed to be deficient and partly compensated for by an increase in the distal aldosterone-dependent reabsorption.

Thus the patient has tubular damage resulting in deficient hydrogen gradient formation and pH regulation, excessive potassium loss and diminished ability to regulate sodium excretion. Other tubular functions, such as concentration capacity reabsorption of glucose, amino acids, calcium and phosphate, are normal, as also is the glomerular filtration rate. In primary RTA potassium depletion is the rule, and simultaneously the capacity for renal sodium conservation is decreased. These disturbances are at least partly corrected by administration of bicarbonate and correction of the acidosis (3). Our patient had a latent RTA associated with hypergammaglobulinaemia and reacted differently to treatment. Hypokalaemia was only seen during alkalosis and disappeared during acidosis. A similar response has been reported in 2 patients with RTA associated with the Fanconi syndrome, involving multiple defects of proximal tubular function (10). The response suggests a less specific tubular lesion than that found in primary RTA. The transport of hydrogen, sodium and potassium ions is probably primarily affected.

Renal biopsy revealed morphological changes indicative of tubular and interstitial nephritis associated with slight reactive changes in the glomeruli. The findings obtained with IF and EM were very much in agreement. Linear staining of the glomerular basement membrane for IgG with focal accentuation in mesangial areas was found. Immunoglobulins in granular form were detected in the tubular cells and lumina. These findings correspond well to the uneven thickening of the glomerular basement membrane and the numerous cytosomes in the tubular epithelial cells revealed by EM. Such cytosomes are known to be associated with protein absorption (1).

Two suggestions can be made concerning the mechanism of the tubular dysfunction in this patient. Firstly the immunoglobulins, after travelling through the glomerular basement membrane into the tubuli, may have caused cellular damage. This



Fig 9 Immunoglobulin as granules within the tubular cells and in the lumina. Kidney section stained with fluorescein-labelled anti-human IgG antiserum.

alternative agrees with the corresponding findings obtained with the IF and EM. Secondly it is possible that the changes observed with the IF technique are only secondary manifestations, and that a primary disease of the interstitial tissue and tubuli is responsible for the tissue damage and tubular dysfunction. This alternative concurs with certain findings produced by EM, viz. destruction of the proximal tubuli and the inflammatory reaction found in the interstitium. The occurrence of lymphocytes between epithelial cells is also suggestive of primary tubular disease. The recent observation that RTA associated with Sjögren's syndrome is only seen in cases which display lymphocytic infiltration of the renal interstitium, while hypergammaglobulinaemia per se does not produce RTA in this syndrome (17), nor in rheumatoid arthritis (7), adds some weight to the latter suggestion.

ACKNOWLEDGEMENT

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ACUTE ERYTHRAEMIA (DI GUGLIELMO'S SYNDROME) AFTER THOROTRAST INJECTION

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Abstract. Fifteen years after an injection of thorotrast typical acute Di Guglielmo erythraemia developed in man aged 65 years. Death occurred few months after the onset of the initial symptoms. Autoradiography revealed signs of alpha radiation in the liver and spleen. The role of thorium dioxide in the aetiology of the disease is discussed.

It is difficult to provide evidence which with merely some degree of probability throws light on the causes of malignant blood disorders in man. The scattered observations made in human pathology will *never have the same conclusive* force as well-planned animal experiments. As long as our knowledge of possible leukaemogenic factors in man is sparse, even sporadic observations will be of value in accumulating additional knowledge. This prompted us to report the following case of acute Di Guglielmo erythraemia which developed 15 years after the patient had been given an injection of thorium dioxide (thorotrast).

CASE REPORT

A 65-year-old man, born May 14 1896, was admitted to the Department of Medicine, Herning Central Hospital, with anaemia in Nov 1961

In 1946 he had been admitted to neurological department with complaint of headache Carotid arteriography with injection of 20 ml thorotrast was performed, but no abnormalities ere revealed, and since then the patient had largely been in good health until the present illness developed.

During the last six months before admission he had felt increasingly tired and eak; his appetite had been poor and he had lost eight. During the last two or three weeks increasing dyspnoea had developed, and easy bruising occurred.

On admission the patient was in poor general health. He as dyspnoeic and deeply anaemic. As the I. globin concentration was 5 6 g 100 ml, blood transfusion was given at once.

Blood smears revealed many large erythrocytes; pronounced anisocytosis and poikilocytosis were present. Numerous erythroblasts, about 8 000 l, were observed. The erythroblasts showed the same morphological abnormalities as are described under the bone marrow Reticulocytes 1.2–2.6%.

Leukocytes 2 600 μl. The differential count revealed 42% mononuclear cells with irregular, somewhat indented nuclei and fine chromatin structure. The cytoplasm was greyish blue with fine dustlike granules. There were 22% juveniles, 7% polymorphonuclears, 4% eosinophils and 25% lymphocytes.

Platelets 5 000–16 000 l. Coombs test was negative. ESR 26 mm/h. Serum creatinine 0 8–1 1 mg/100 ml; serum bilirubin 2 mg/100 ml. Prothrombin concentration 90%. Urobilinuria was present.

The bone marrow as hypercellular, greatly dominated by cells belonging to the erythropoiesis. The differential count showed: myeloblasts 1%, promyelocytes 1%, neutrophil myelocytes 2%, neutrophil juveniles 4%, polymorphonuclears 1%, plasma cells 1%, lymphocytes 1%, basophilic erythroblasts 44%, polychromatic and orthochromatic erythroblasts 45%. The erythroblasts showed severe morphological abnormalities. They were mainly very large. In many of the cells there were 2–3 nuclei, occasionally 4 (Fig. 1). The nuclei were often bizarre in form, indented, elongated and often twisted. The chromatin structure as distinct, with great contrast between chromatin and nuclear sap (Fig. 2). Retarded maturation of the nucleus seemed to be present, the chromatin structure remaining delicate, hile the cytoplasm had reached the polychromatic stage. I large number of the cells the cytoplasm was fairly abundant as compared with the size of the nucleus. The granulopoiesis seemed to be somewhat hypoplastic The granulation of the myelocytes was finer than normal. Only relatively few megakaryocytes were present in the smears.

Course

A total of seven blood transfusions of 500 ml were given, resulting in haemoglobin level of 7–8 g/100 ml. I addition, prednisone and antibiotics were administered.



Fig. 1 A three-nucleated giant erythroblast typical of Di Guglielmo's syndrome.

condition remained poor. The patient was dyspnoeic and had slight fever. Death occurred 22 days after admission, with signs of pulmonary oedema.

Autopsy

Gross appearance. The *liver* was small, slightly lumpy due to fibrous, irregular thickening of the capsule; the parenchyma was soft, without infiltrations or changed pattern. The *spleen* was small, atrophic and soft. The cut surface showed coarse connective tissue pattern, but no definite infiltrations. The *kidneys* were large, slightly swollen, very soft and pale. They were of normal shape with smooth surface; there were no infiltrations. The *bone marrow* of the vertebrae was soft and hyperplastic, but without haemorrhages or infiltrations. There was no enlargement of the cervical, thoracic, abdominal or other regional *lymph nodes*.



Fig. 2. T megaloblastoid erythroblasts with distinct nuclear chromatin pattern. In the centre a myeloblast, and below pathological promyeloblast.

The *heart* was dilated and slightly hypertrophic. The myocardium was pale and soft without infiltrations. The *lungs* were pale, slightly mottled, of normal shape and size, and markedly oedematous. A few scattered pleural adhesions were noted, but otherwise the serous membranes were normal, in particular no haemorrhages were present.

The *intestinal mucosa* was pale, with some tiny haemorrhages; but infiltrations and ulcerations were absent.

The pale *vesical mucosa* showed scattered minute haemorrhages.

Histological examination

The *liver* did not show any definite increase in connective tissue or well-defined infiltrations, but few large, binucleated erythroblasts were present in the sinusoids. The parenchyma was normal, apart from slight, typical fatty degeneration. Scattered everywhere in the hepatic tissue there were grey or brownish particles, either in small amounts in the Kupffer cells or in larger accumulations between the liver-cell trabeculae. Staining for iron was negative. The hepatic tissue did not reveal any angiomatous changes.

The normal structure of the *spleen* was completely changed, with disappearance of the follicles and distinct wide trabeculae of connective tissue. Massive, diffuse extramedullary myelopoiesis was present, dominated by large, irregular erythroblasts, often with 2 3 irregular elongated nuclei with slightly changed chromatin structure and widely varying staining of the cytoplasm. Large amounts of particles similar t those observed in the liver were present everywhere in the tissue (Fig. 3).

Tissue from both kidneys showed no definite abnormalities, in particular there were no infiltrations, extramedullary myelopoiesis or deposition of particles.

Sections of the spinal *bone marrow* revealed highly hypercellular marrow completely dominated by large, ir regular erythroblasts with sparse and immature granulopoiesis. Moderate deposits of particles were noted.

Autoradiography

Sections from the liver and spleen showed intense blackening of the films, corresponding to the deposited par ticles, and typical traces of alpha particles, just as found on deposition of thorium dioxide (Fig. 4).

DISCUSSION

Our patient exhibited the clinical manifestations which are typical of the Di Guglielmo ery (10 11 12, 13). There were severe anaem jaundice and thrombocytopenia, and the dise led rapidly to death.

The haematological picture was diagnostic the disease, with the occurrence of numerous erythroblasts in the blood and a bone marrow ir which these cells constituted more than 75%. A feature which is of special importance in the diagnosis of the disease is the morphology of the erythroblasts (2, 23). In our case these cells had

the megaloblastoid appearance which is characteristic of the Di Guglielmo syndrome, with retarded maturation of the nucleus, abundant cytoplasm and a distinct chromatin pattern with a sharp contrast between the chromatin network and the nuclear sap. In addition it was typical of the cells that they were large and contained indented and twisted nuclei, sometimes several nuclei, but still with the same distinct chromatin pattern.

It was not difficult in this case to exclude the disorders which must be considered in the differential diagnosis in such a situation, viz. megaloblastic anaemias and a severe haemolytic crisis.

Haematologically the case was thus characterized by the pronounced changes in the red blood picture. The number of leukocytes in the blood was normal, or rather slightly subnormal. The white blood picture was marked by mononuclear cells with a finely granulated cytoplasm, cells which we interpreted as promyelocytes and myelocytes.

Many authors, including Di Guglielmo, have made a point of distinguishing erythraemia from erythroleukaemia. In the latter disorder a fair amount of immature myeloid cells will be present in the blood together with the erythroblasts, whereas leukaemic leukocytes should not occur in erythraemia. However the occurrence of reticulo-endothelial cells are often mentioned in the literature on the Di Guglielmo erythraemia, presenting as mononuclear cells with an irregular shape of the nucleus, a varying chromatin pattern and a dustlike cytoplasm. These cells seem to be similar to those seen in myelomonocytic leukaemia, cells which by many authors are considered to be pathological promyelocytes and myelocytes (24). It was such cells we found in the blood of our patient.

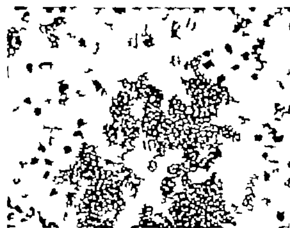


Fig. 4 Autoradiograph from section of spleen showing fine tracts radiating from the thorotrast pigment, suggestive of alpha radiation.

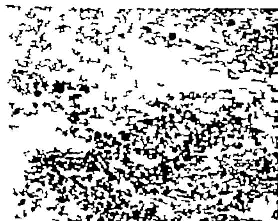


Fig. 3 Section of spleen showing extramedullary hematopoiesis and deposits of thorium dioxide.

On the other hand a sharp distinction between erythraemia and erythroleukaemia will be less reasonable if the monocytoid cells in the Di Guglielmo erythraemia are conceived of as immature cells of the granulocytic series. It also seems to be more common to assume that the two disorders are actually different stages of development of the same disease. Dameshek and Gunz (6) reported that, in their experience, it is not uncommon in the acute Di Guglielmo erythraemia to find some immature cells of the granulocytic series in the blood of the patients.

The acute Di Guglielmo erythraemia should presumably be conceived of as a special incipient form of myeloblastic leukaemia. Examples are on record in which the disease is transformed into a morphologically typical myeloblastic or myelomonocytic leukaemia if the patients live long enough (6, 7, 18). Dameshek and Gunz (6) reported that the reverse development may be seen in rare cases. One of us (B.-M.) has also had an opportunity to observe a case of typical myeloblastic leukaemia which after some time developed into a morphologically characteristic Di Guglielmo erythraemia. In this connexion it should be mentioned that it is not rare in typical

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MYELOFIBROSIS WITH MYELOID METAPLASIA AND PANCYTOPENIA AFTER THOROTRAST INJECTION

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Abstract. Twenty-two years after an injection of thorotrast, myelofibrosis with extramedullary metaplasia of the spleen and lymph nodes developed in a man aged 50 years. Pancytopenia with erythroblasts and poikilocytes in the blood was present. The clinical picture was characterized by anaemia, infections and haemorrhages, which ultimately led to death. The case is remarkable owing to the absence of splenomegaly. Both the spleen and the lymph nodes were the site of marked follicular atrophy presumably evoked by radiation from the deposited thorium dioxide, which was also present in the liver and bone marrow. This radioactive material, which is assumed to have induced the myeloproliferative disorder, is also likely to have accentuated the pancytopenia. It is pointed out that some of the cases which are described in the literature as aplastic anaemia after thorotrast injection had morphological changes in the red blood picture of the same type as in myelofibrosis. This diagnosis may be overlooked if the thorium radiation induces splenic atrophy instead of the splenomegaly which is otherwise characteristic feature.

The great sensitivity of the haematopoietic tissue to ionizing radiation has been known since the beginning of this century when Heinecke (8) published his basic studies. In 1922 Fabricius-Møller (7) demonstrated that X-ray irradiation of guinea ... resulted within a few days in thrombocyto- ... referable to bone-marrow injury. The ensuing haemorrhage led to death in the experimental ...s. This phenomenon was also observed in victims of the explosion of the atomic bombs in Hiroshima and Nagasaki in 1945 (5, 10, 13). A voluminous literature is available on the haematological aspects of ionizing radiation, and the ... is therefore referred to the survey edited by Szirmai (20).

The cellular damage which occurs when haematopoietic tissue is exposed to ionizing radiation is of the same quantitative nature, whether caused by gamma, alpha or beta rays, or by neutrons. An aplastic anaemia, the severity of which depends on the size of the dose, can be induced in experimental animals (18).

The present extensive use of radioactive material for purposes useful within many fields of modern life is governed by strict rules and calls for minute caution. Nevertheless cases of aplastic anaemia caused by exposure to radioactive substances are known. Apart from the intended destruction in Hiroshima and Nagasaki, such cases have occurred by accident. Cases are on record in which scientists have been exposed to radioactivity due to leakage from a cyclotron, or in which some Japanese fishermen were exposed to fall-out of radioactive ashes while at sea 140 km from the hypocentre of the nuclear test explosion at Bikini in 1954.

However, some cases of aplastic anaemia occurred before the extent of the harmful effects of ionizing radiation were fully realized, for example in some of the pioneers of radiology (4) and in luminous watch-dial workers exposed to radium (14). The introduction of thorotrast as a contrast medium in angiography must be included under such accidents. This medium, which consists of a 25% colloidal solution of thorium dioxide, was from a certain point of view well suited for its purpose: it gave good contrasts, had only a slight tendency to cause arteriospasms, and no immediate toxic effects were observed. However, thorium oxide is radioactive with a very long half-life of 1.4×10^{10} years. As it is not excreted, but stored wherever reticuloendothelial tissues are present, a person in whom this radioactive material has been employed will be exposed to the radiation for the rest of his life. It is true that this radiation takes the form of alpha rays, which have

a power of penetration of only a few μ. Nevertheless, it cannot be excluded that it may have a biological effect owing to its wide distribution in the body After a lapse of 20–25 years its use was discontinued, partly because of the considerations just mentioned, and partly because investigators called attention to pathological conditions which might be ascribed to the action of thorium.

Below we report the occurrence of severe pancytopenia and myelofibrosis with myeloid metaplasia 22 years after arteriography with thorotrast. In connexion with this study we have collected some similar cases from the literature and, on this basis, we discuss the possible aetiological importance of thorium dioxide in the disease

CASE REPORT

A 50-year-old man, born Dec. 21 1911 was admitted to the Department of Medicine II, Aarhus Amtssygehus, because of protracted fever in Oct. 1961

When the patient was 24 and 28 years old, some haemangiomata on the right side of the face had been removed. In 1939 he had been subjected to right-sided carotid arteriography with injection of 20 ml thorotrast, because a similar intracranial process was suspected due to the fact that radiographs had revealed a shadow in the right occipital region. However the arteriography did not show any abnormality but pneumo-encephalography revealed some dislocation of the posterior horn due to a calcification in the right occipital region.

In 1957 the patient was admitted to this department with a depressive neurosis. His Hb concentration on admission was 16.4 g/100 ml. The ESR was 4 mm/hour The white blood cells were not studied.

A month before the present admission the patient was treated by his dentist for a periapical abscess. Since then he had felt tired and weak. He had had persistent fever in spite of antibiotic therapy at home. During the last fortnight before admission he had had daily episodes with chills.

On admission he had slight fever, but his general condition was relatively good. Otherwise clinical examination did not reveal any abnormalities; in particular there was no enlargement of the spleen, liver or lymph nodes, or haemorrhages into the skin. Radiography of the skull revealed periapical abscesses of several teeth. Despite treatment of these abscesses the body temperature remained slightly elevated.

Additional radiographic studies revealed accumulations of contrast medium typical of thorotrast. Shadows were seen along the carotid sheath on the right side of the cervical spine, in the regional lymph nodes across the abdomen and, particularly in the spleen.

The Hb level was 12.3 g/100 ml; the leukocyte count 3 320, with 52% neutrophils, 2% eosinophils, 45% lymphocytes and 1% monocytes. Reticulocytes 0.2–0.4%. As assessed in smears, the number of platelets seemed normal. ESR 55 mm/h. A series of blood cultures yielded no growth.

During the next two months the general condition gradually deteriorated. Pancytopenia developed. The Hb concentration fell to 6.4 g/100 ml; the leukocyte count went down to 350/μl with complete disappearance of neutrophils, and the platelet count fell from 160 000 to 20 000/μl. In the smears the erythrocytes showed anisocytosis, poikilocytosis and polychromasia, and few erythroblasts were noted. Repeated bone-marrow punctures yielded no material A biopsy specimen secured from the iliac crest showed that many of the marrow spaces were necrotic only towards the bone trabeculae were there small amounts of cellular marrow which contained erythroblasts, myeloblasts and myelocytes, but no polymorphonuclear leukocytes. In some areas the connective tissue was increased and some granular deposits were observed. Fever was still present and the ESR had risen to 100 mm/h.

In addition to antibiotic treatment the patient was given blood transfusions. With 500 ml blood infused about every fifth day the haemoglobin level was kept at about 8 g/100 ml. Attempts were made to reduce the need for blood transfusions, first by treatment with androgens (100 mg sublingually daily) and later by supplementing the androgen therapy with prednisone 20 mg daily but the therapeutic efforts were ineffective. In the terminal phase of the disease large perianal abscess developed, accompanied by a tendency to severe haemorrhages and a steep fall in the haemoglobin level. Death occurred five months after admission.

Histological Findings after Autopsy

Lymph nodes

The mediastinal lymph nodes showed pronounced follicular atrophy with absence of germinal centres. Extramedullary haematopoiesis with cells from both the granulopoiesis and erythropoiesis was observed, in addition some megakaryocytes were present. In the abdominal lymph nodes the lymphoid tissue was displaced by fibrous connective tissue. Distinct deposition of thorotrast pigment was disclosed.

Spleen

The follicles were markedly atrophic. Accumulations of greyish brown coarse granules of thorotrast pigment were seen in the trabeculae, around the vessels and scattered in the pulp. This pigment was surrounded by considerably increased amounts of connective tissue. In addition there was extramedullary haematopoiesis, but both the granulopoiesis and thrombopoiesis were sparse while numerous erythroblasts were noted. These were of varying size some of the large erythroblasts had large, atypical and often somewhat indented nucleus, or several nuclei were present in some of them.

Bone marrow

Most of the marrow consisted of finely fibrillar loose tissue with fibroblast-like cells. Some areas revealed

Table I. *Pancytopenia after thorotrast*

Authors	Dose (ml)	Latent period (y.)	Died	Bone marrow	Blood
Spier et al., 1947	?	9	+	?	Poikilocytosis
Birkner 1948	70	17	?	?	?
Schmidt et al., 1950	?	10	+	Pathological erythroblasts	?
Rotter 1951	10	3	+	Pathological erythroblasts	Erythroblasts
Moeschlin et al., 1953	30	6	+	Pathological erythroblasts	Erythroblasts. Poikilocytosis
Hieronymi and Rand-köhler 1953	?	11	+	?	Leuk. 1900. A few "paramyeloblasts
Johansen, 1954	54	11	?	?	?
Duane, 1957	?	14	?	?	
Bestrup-Madsen and Nordentoft Jensen, 1970	20	22	+	Pathological erythroblasts	Erythroblasts. Poikilocytosis

hypercellular foci with brisk haematopoiesis. I these foci there are few myelocytes, no polymorphonuclear granulocytes, but many erythroblasts hich exhibited the same nuclear abnormalities as are described under the spleen.

Liver

Abundant storage of thorotrast pigment was observed, especially in the portobiliary spaces.

Autoradiography

Films exposed to sections from the liver spleen and lymph nodes showed numerous fine dark tracts radiating from all sides of the thorotrast pigment, suggestive of alpha radiation.

DISCUSSION

Clinically the case described was characterized by the manifestations of pancytopenia, symptoms of anaemia, and a tendency to infections and haemorrhages.

Twenty-two years previously the patient had received a thorotrast injection. The contrast medium was stored in the haematopoietic organs and in the liver and the follicular atrophy which is typical of exposure to thorium was present. Autoradiography revealed alpha radiation. It must be strongly suspected that the internal radiation by thorium was the cause of the haematological disorder The presence of hypoplasia of the bone marrow with resulting pancytopenia is in good agreement with the experience gained in experimental animals and in human beings who have been exposed to intense radioactivity The occurrence f such manifestations after injection of thorium dioxide has also been demonstrated in rabbit experiments (12).

Already in the study of the blood smears it was found that our case differed from classical aplastic anaemia, in that erythroblasts were present and the mature erythrocytes showed distinct poikilocytosis. These findings combined with proliferation of connective tissue in the bone marrow and the presence of myeloid foci in the spleen and *lymph nodes were in favour of a diagnosis of* myelofibrotic anaemia with myeloid metaplasia. The severe pancytopenia, which was a conspicuous feature in the clinical picture, is not incompatible with the diagnosis of myelofibrosis, even though its occurrence is relatively rare. In a series of 56 cases Andreasen (2) found that the vast majority of the patients had normal or only slightly reduced leukocyte and platelet counts in the quiescent phase of the disease. However in the terminal phase about 10% of the patients had leukocyte counts under 1 000/μl, and severe thrombocytopenia developed in an appreciable number of the cases.

However in our patient an important component was absent in the clinical picture, viz. *splenomegaly* It is likely that the internal radiation from thorium, with its typical destructive action on lymphoid tissue, prevented the development of this otherwise conspicuous feature of the disease. Even though pancytopenia is compatible with myelofibrosis, it is likely that this condition had been accentuated by the thorium radiation.

The possibility of a causal relationship between radioactive exposure and the occurrence of myelofibrosis became of topical interest after it was reported that this disease developed in ten Japanese who had been exposed to the atomic bomb (1). The mean distance of the victims from the hypocentre was 1 190 m, and there was a correlation between the incidence of myelofibrosis and the distance from the hypocentre. Moreover myelofibrosis was approximately five times more frequent in exposed than in non-exposed persons. Anaemia, a typical blood picture of leuko-erythroblastosis, splenomegaly and extramedullary haematopoiesis were observed in all cases.

Johansen (11) reported a case of myelosclerosis which occurred seven years after a thorotrast injection. In Table I we have listed cases which have been described under the diagnosis of aplastic anaemia after thorotrast injection (3 6, 9 11 15 16, 17 19). It will be seen that some of these cases exhibited changes similar to those observed in our patient, viz. poikilocytosis and erythroblasts in the blood. For these reasons the clearcut diagnosis of aplastic anaemia must be doubted. It must be emphasized that myelofibrotic anaemia may be overlooked if the thorium radiation gives rise to splenic atrophy instead of splenomegaly which is characteristic of the disease. In the presence of pancytopenia in patients who have been exposed to thorotrast radiation it will therefore be reasonable to look very carefully for extramedullary foci.

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INTRAVASCULAR COAGULATION AND ACUTE RENAL FAILURE IN A CHILD WITH MYCOPLASMA INFECTION

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Abstract. Report of a 3-year-old girl with an acute disease with pneumonia, haemolysis, uraemia and intravascular coagulation. She died after one month in spite of normalisation of coagulation and renal function. The cause of death was Candida septicaemia. Serological evidence of Mycoplasma infection was found.

In recent years disseminated intravascular coagulation has been reported to play a role in various apparently different pathological processes (1., 17 1 22, 24 30, 31 33 36). Conditions in which disseminated intravascular coagulation has been considered an important pathogenetic factor are thrombotic thrombocytopenic purpura, haemolytic uraemic syndrome, renal cortical necrosis, malignant hypertension, liver cirrhosis and hyaline membrane disease Shwartzman phenomenon and shock of various aetiology (33). Microthrombi may occur in any of the organs, but are often seen in the kidney (22) Renal failure with transient oliguria, anuria for several days followed by diuresis, or irreversible anuria and death in uraemia are common in disseminated intravascular coagulation.

This paper concerns a fatal case in a 3-year old girl who developed a coagulation disorder compatible with a diagnosis of acute disseminated intravascular coagulation and extensive gangrene of the lower legs and severe renal failure. The patient had serological evidence of mycoplasma infection but also showed signs of haemolytic uraemic syndrome. After treatment of the patient with heparin and haemodialysis the renal failure and the coagulation disorder improved but her general condition deteriorated and she died from Candida septicaemia.

CASE REPORT

The patient was a 3-year-old girl (weight about 12 kg) who had always felt well except for occasional respiratory tract infections. On Feb 26, 1969 she fell ill with fever vomiting and increasing respiratory distress On March 1 she was admitted to the Paediatric Department, Malmö General Hospital.

Examination on admission revealed tachycardia, tachypnoea, peripheral cyanosis and somnolence. Chest X-ray demonstrated right-sided pneumonia with atelectasis of the right upper lobe. She was treated with benzylpenicillin and Keflin®. But the child deteriorated, with increasing circulatory insufficiency and tachypnoea. On March 2 she was therefore transferred to the unit for respiratory failure at the Department for Infectious Diseases. On admission her condition was marked by prostration, shallow respiration, cyanosis of the lips and severe peripheral cyanosis with blue-black discoloration of the feet. Petechiae were seen in the distal parts of the legs. Temp. 39.4°C, pulse 140 min, B.P 100/60, and respiratory rate 80/min. During the night the child had generalised convulsions and such respiratory distress that she was connected to an IPPB respirator No focal neurological signs were noted. The excretion of urine decreased and finally ceased, and on March 6 the girl was treated by haemodialysis. Such treatment was given altogether eight times.

Owing to signs of intravascular coagulation, treatment with heparin and fresh blood was started already on March 3 ACTH was also given.

The patient was first treated with Kanamycin® and Terramycin® and later with small doses of Gentamycin®. Culture of bronchial secretion was initially negative but on March 17 revealed growth of Candida, as did culture of blood. Treatment was then extended to include amphotericin B.

Further course

Gangrene of the legs developed and progressed proximally (Fig. 1). Renal function gradually improved, and on March 17 the patient produced more than 150 ml of urine. The platelet count increased to almost normal, but chest X-ray showed deterioration of the condition of



Fig. 1. Gangrene of the feet.

the lungs, and on March 19 examination revealed right sided pneumothorax due to perforation of an abscess that had developed in the meantime. On March 1 profuse rectal bleeding supervened and a tendency to shock was controlled by infusion of fresh blood, but the septic condition persisted. The girl was treated with erythromycin and Mycostatin®, but chest X-ray showed further deterioration of the lungs and the patient died on April 7.

Laboratory studies

The hite blood cell count on admission was 4 00 /µl. It rose rapidly to more than 30 000 /µl. Differential count showed shift to the left with many immature cells and toxic picture of the leucocytes. Hb (March 3, first value) 6.9 mg/100 ml, red blood cell count .8 mill./µl, reticulocytes 10 000/µl. Smear of bone marrow from crista iliaca showed my lopoetic hyperplasia but no evident increase of erythropoesis or megakaryocytes. No "burr cells" were seen. ESR on admission 5 mm/1 h. Electrophoresis demonstrated severe hydraemia, highly acute inflammatory process and elimination of haptoglobin. No hyperbilirubinaemia. Serum creatinine on March 3 as .6 mg/100 ml, after which it aried between 1.2 and 3.3 mg/100 ml.

Cultures of blood and sputum ere made only after institution of antibiotic therapy. The blood initially gave no growth and the sputum ga growth only of Candida and no significant bacteria. Later on thorough search was made for bacterial or viral agents. The only positiv finding was complement fixation test for Mycoplasma pneumoniae antibodies, hich showed titre increase from 1 8 to 1 64. Examination of the cerebrospinal fluid showed nothing pathological.

Coagulation studies

Platelet counts er made by the method of Bjorkman ( ). The bleeding time as determined by the method of Duke with standardized haemolets (Dade Reagent, Inc., Miami, Florida, USA). Determinations of the coagulation time in glass and plastic tubes, recalcification time, factor VIII one-stage prothrombin time, prothrombin, factor VII and factor X (Owren P & P test) and factor



Fig. 2. Small subcutaneous artery from leg with endothelialized fibrin deposit. 290.

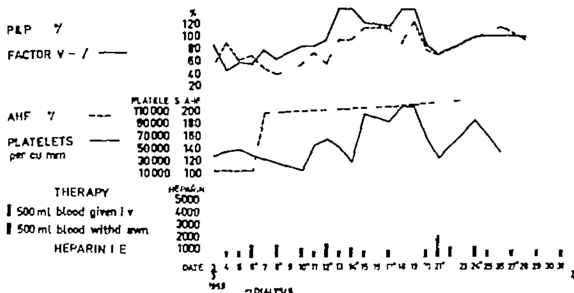


Fig. 3. Platelets, AHF, P & P and factor V during the course of the disease.

V were made by methods described earlier (26). Fibrinogen was determined in the way described by Nilsson and Olow (27). Only fibrinogen values for blood collected with EACA are given. Plasminogen was measured by an immunochemical method (8). The α_2-macroglobulin (α_2M) concentration was determined by the method of Ganrot (6). Fibrinolytic activity of plasma and resuspended euglobulin precipitate was measured on unheated bovine fibrin plates, as described by Nilsson and Olow (28). Fibrinolytic split products were determined in serum and in urine by the immunochemical method of Nilsson (25). The analyses were performed on serum samples obtained from blood collected in tubes containing EACA.

Results of coagulation studies

Coagulation analyses were performed immediately on admission (Figs. 3 and 4). The patient had thrombocytopenia and low levels of P & P and factor V. The fibrinogen level was 0.28 g/100 ml and the AHF level 99 %. The values of α_2M and plasminogen were low. Tests on fibrin plates showed no fibrinolytic activity in the blood. The amount of fibrinolytic split products in the circulating blood was abnormally large. Split products were also demonstrated in the urine.

Treatment with heparin was started two days after admission to the hospital and exchange transfusions were given for substitution of platelets and coagulation factors. The coagulation time in glass tubes was kept between 20–60 min. The coagulation status remained largely unchanged in the first few days (Figs. 3 and 4). On the 4th day the level of factor VIII and factor V started to rise and after 8 to 11 days also the P & P and platelet count increased. The concentration of fibrinolytic split products in the serum rose, while that in the urine fell. No fibrinolytic activity was ever demonstrated in the blood during the disease. The plasminogen increased only slightly and α_2M remained low throughout. After 14 days the platelet count, factor V and P & P were normal and the fibrinogen and factor VIII were increased. After 3 weeks, by which time renal function had improved, only traces of split products could be demonstrated in the serum and urine.

Autopsy

Gross findings. Dry gangrene on fingers and toes. Deep ulcerations on medial aspect of the lower legs. Petechiae on the skin. Some subendocardial bleedings and mural thrombosis in the right auricle and the left ventricle. No gross thrombi in systemic arteries or veins or in the pulmonary arteries. The right pleural cavity was lined with thick fibrinous masses. The lungs were atelectatic with alternating pale and haemorrhagic areas. There was tracheostomy and ulceration in the tracheal mucosa. The ventricular mucosa exhibited many shallow ulcerations. The kidneys were enlarged and showed many small abscesses throughout the parenchyma. No infarction or signs of necrosis in cortex or medulla. The brain was swollen and its parenchyma contained haemorrhagic foci.

Microscopical findings. Signs of septicaemia were seen.

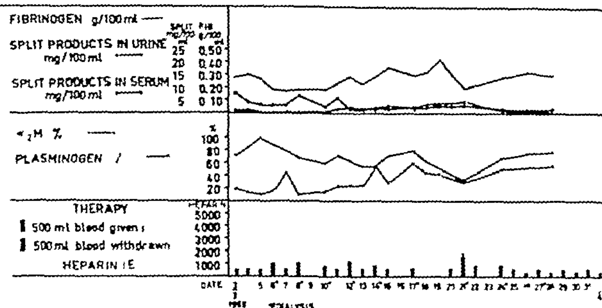


Fig. 4. Fibrinogen, split products, α_2M and plasminogen during the course of the disease.

The kidneys, the myocardium and the brain showed numerous small abscesses containing Candida.

The lungs exhibited extensive changes. There was a fibrinous exudate in alveoli and bronchioli. Many small arteries and veins contained fibrinous thrombi and nearby small irregular necrotic patches. Staining for fungi revealed no organisms in the lungs. The morphology of the kidneys was distorted by the septic changes. Besides the macro-abscesses there were signs of diffuse, acute pyelonephritis. Some small thrombi were found in the vessels contiguous to the abscesses, but no evidence of diffuse vascular or glomerular thrombosis. In the spleen many small hyaline thrombi were seen. Sections from the dermis of the lower leg showed many partly endothelialized fibrin strands traversing the lumen of small arteries and arterioles (Fig. 2). The pancreatic parenchyma showed diffuse inflammation with fibrosis.

Culture of necropsy specimens of the kidneys and the lungs gave growth of Candida albicans but no significant growth of bacteria. An attempt at isolation of virus from the brain proved unsuccessful.

DISCUSSION

In this patient the clinical picture with gangrene of the toes, oliguria and anuria, haemolysis, mental confusion, respiratory distress and shock strongly indicated the presence of acute disseminated intravascular coagulation. The results of coagulation analyses, low platelet count and decreased levels of the prothrombin group and factor V were also compatible with such a diagnosis. The fibrinogen and factor VIII which are decreased in consumption coagulopathy were normal. Factor VIII and fibrinogen are increased in all reactive processes, and in view of the severe septic condition in this patient the normal levels might therefore be regarded as low. Fibrinolytic split products were demonstrated both in the serum and urine. Tests on fibrin plates showed no fibrinolytic activity indicating that the fibrinolytic split products derived from local dissolution of fibrin deposits. Rayner et al. (34) and Larsson et al. (16) have produced evidence for the simultaneous occurrence of split products in the serum and the urine being a sign of renal disease and indicating the presence of local fibrin deposits in the kidney. The low plasminogen level can be explained by consumption in the secondary fibrinolytic process. The α_2M was markedly decreased (the normal level for 3-year-old children is about 250%). α_2M can bind both plasmin and thrombin (7) and be decreased in conditions associated with systemic fibrinolysis as well as in conditions associated with intravascular coagulation. The changes observed in the coagulation and fibrinolytic systems in this case could thus be attributed to disseminated intravascular coagulation and secondary fibrinolysis. And autopsy demonstrated fibrin thrombi of small vessels in the lungs, spleen and skin.

Several mechanisms may have been involved in the induction of intravascular coagulation in this case. The patient might have originally had a haemolytic uraemic syndrome or thrombotic thrombocytopenic purpura, diseases described as being associated with intravascular coagulation (3 9 22). She did have haemolytic anaemia, thrombocytopenia, acute renal failure, haemorrhagic diathesis and cerebral symptoms. But repeated examination of blood films revealed no "burr cells It has been demonstrated that disseminated intravascular coagulation may cause haemolysis the haemolytic process in this patient may therefore not be the cause, but rather the effect, of the intravascular coagulation (23). Gilchrist et al. (10) have recently reported a series of patients with *haemolytic uraemic syndrome. They point out* that these patients have thrombocytopenia but normal or high levels of fibrinogen, prothrombin and factor VIII, and thus no signs of disseminated intravascular coagulation. Because of the septic picture it was difficult to evaluate the morphological changes. The findings in the kidneys were, however not of the type usually seen in thrombotic thrombocytopenic purpura (arteriolar thrombosis with microaneurysms and diffuse glomerular changes), and no renal cortical necrosis was found (as described in the haemolytic uraemic syndrome). Taken together, the observations made in this patient do not provide convincing evidence of the presence of a haemolytic uraemic syndrome, but such a diagnosis cannot be excluded

One might also imagine that the disseminated intravascular coagulation in our patient was a link in a generalized Shwartzman reaction induced by bacterial endotoxins, for example (12, 21). The clinical picture on admission was that of a septic shock with high grade fever leucocytosis and pulmonary lesions. The other clinical evidence, including intravascular coagulation, is also compatible with the Shwartzman reaction (13). Unfortunately no blood samples for cultures were obtained before institution of antibiotic therapy But afterwards an extensive search was made for virus and bacteria. The only positive finding was a significant titre increase from 1 8 to 1 64 in the complement fixation test for Mycoplasma pneumoniae antibodies. There was no reason to assume that this increase was due to previous blood transfusion. The occurrence of some type of Gram-negative or Gram-positive septicaemia in this patient cannot be excluded, but it seems more likely that the Shwartzman-like reaction and the disseminated intravascular coagulation were provoked by the Mycoplasma infection. In 1947 Noran et al. (29) put forward the hypothesis that the neurological complications of the infection with Mycoplasma were the result of intravascular clotting due to release of substances from damaged lung tissue. A review of the central nervous system complications of Mycoplasma infection has been made by Yesnick (37). Recently two fatal cases of infection with Mycoplasma pneumoniae have been reported. Maisel et al (19) described a 70-year-old woman with pneumonia who died 21 days after onset of symptoms. She had haemolytic anaemia and signs of hypercoagulability and autopsy showed widespread thromboemboli and infarctions. The fatal case reported by Sterner and Biberfeld (35) also showed thromboemboli. In none of these cases were detailed coagulation studies performed. Despite lack of convincing evidence it seems possible that the disseminated intravascular coagulation in our patient was triggered off either by toxins from Mycoplasma pneumoniae or by thromboplastin released from the damaged lungs.

Our patient was treated with heparin, exchange transfusions, haemodialysis, Actocortin® and various types of antibiotics. Coagulation gradually became normal. Whether this was an effect of the treatment with heparin is unknown. The declining concentration of split products, however suggests that the deposition of fibrin and the following dissolution of fibrin deposits decreased. Also renal function improved and diuresis returned. Despite normalization of the coagulation factors and of renal function, the fatal issue could not be warded off. Heparin is often recommended in the treatment of diseases associated with acute disseminated intravascular coagulation and in the haemolytic uraemic syndrome (1 4 10, 20) but the results obtained have been inconsistent and the control material inadequate. According to some workers (10 15 32) heparinization with or without dialysis will reduce the mortality in children with haemolytic uraemic syndrome. On the other hand other workers have reported no success with heparin treatment in haemolytic uraemic syndrome (11 14). Leach (17) recommends treatment not only with heparin, but also with streptokinase in cases with disseminated intravascular

coagulation, impaired peripheral circulation and shock in order to dissolve the fibrin deposits. Cronberg and Nilsson (5) described a successfully treated case of acute pneumococcal sepsis and typical lesions of intravascular coagulation. Their patient was not given heparin but only Macrodex® which is known to inhibit platelet adhesion. In our patient heparin did not prevent progression of the disease. It is possible that combined treatment with heparin and Macrodex® and/or heparin and streptokinase would have been more effective in the prevention and dissolution of microthrombi in our case. Furthermore, we feel that intravascular coagulation is only a symptom in diseases like this, and that the intravascular coagulation need not have anything to do with the shock and the fatal outcome. Lerner et al. (18) showed that intravascular coagulation and endotoxin shock are independent manifestations of endotoxaemia.

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OBSERVATIONS DURING TREATMENT OF CYSTINURIA WITH *D*-PENICILLAMINE

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Abstract. Three patients with severe stone-producing cystinuria have been treated with *d*-penicillamine for altogether 68 patient-months. The treatment was effective in all instances, but serious complications ensued in two patients. One patient acquired a severe nephrotic syndrome and laboratory signs of systemic lupus erythematosus. This patient is probably the first described with both these complications, and possibly also the first described cystinuric developing SLE from penicillamine. Another patient used the drug throughout pregnancy and gave birth to a child with a generalized connective tissue defect, probably caused by penicillamine dissolution of fetal collagen. This patient seems also to be the first described of the kind. Before *d*-penicillamine is used in the therapy of cystinuria, one should ascertain that other measures have been used to their full extent. Treatment with *d*-penicillamine requires meticulous control in order to detect complications at the earliest possible stage.

Cystinuria is an inborn error of metabolism characterized by a defect of the transport system in kidneys and intestine for the four amino acids cystine, lysine, arginine and ornithine (13). Formation of urinary cystine calculi secondary to the increased cystine excretion is the single clinical manifestation of the defect. Stone-producing cystinuria has a prevalence of about 1 per 200 000 population (1), and cystine stones constitute about 1% of all urinary calculi in adults (22) and about 5% among children (9). The importance of the disease is, however, greater than shown by these figures, since cystinurics in general are more severely affected than patients with other kinds of stone disease. About 25% of patients with stone producing cystinuria die in uremia secondary to stone formation and pyelonephritis, and many undergo repeated operations. The mean life span is consequently considerably reduced (1).

The cornerstones in the conservative treatment of cystinuria are an increased fluid supply and alkalinization of the urine to pH above 7.5 (4). Both the high urine flow and the high urinary pH must be maintained throughout the 24 hours. In order to achieve this the patient must wake up regularly each night for the intake of fluid and alkali and the urinary pH must be regularly controlled. In some patients a methionine-poor diet may help considerably in keeping them stone free (24). Usually this regimen proves effective, but in some cases the disease progresses despite all these precautions. Therefore the introduction of penicillamine in the treatment of cystinuria by Crawhall et al. in 1963 was an important advance (3). Penicillamine and cystine form a disulfide complex which is about 50 times as soluble in water as cystine. The complex is excreted and the content of free cystine in the urine is thus reduced (5, 18). Since then, several reports have confirmed that the drug is effective in prophylaxis and treatment of cystine stones. Even staghorn stones can be dissolved (2, 16, 20). During the same period, however, several complications following penicillamine treatment have also been reported. The exact indications for the use of the drug have therefore not been established.

This report describes three patients with severe cystinuria treated with *d*-penicillamine, illustrating both the effectiveness and the hazards of the treatment.

CASE REPORTS

Case 1

Male, born 1940. No history of relevant familial diseases. From 1966 he had severe psychical disturbances, with depression and two suicidal attempts. In June 1966 he passed his first urinary stone, with similar episodes occurring in Nov. 1966 and Aug. 1967. On the last oc-





Fig. 1 Case 1 (*a*) Feb. 1969 Abdominal flat film showing cystine stone in the middle calyx group of the right kidney (*b*) Sept. 1969 The same region. The stone has disappeared.

cation left-sided ureterolithotomy was performed and chemical analysis of the removed stone established the diagnosis of cystinuria. This was later confirmed by qualitative amino-acid chromatography and cyanide-nitroprusside tests. *The cystine content in urine was 2.8 μmol/mg creatinine* (normally below 0.05 μmol/mg). Fluid intake was forced and bicarbonate was given. However new ureterolithotomies had to be performed on the left side in June and Nov 1968.

Therefore in March 1969 *d*-penicillamine was started with dose of 500 mg three times daily. Since then and till Aug. 1970 the course has been entirely uncomplicated. No symptoms of stone formation have occurred. In spite of constantly positive cyanide-nitroprusside test re sidual stone on the right side has been chemically dissolved (Fig. 1). Qualitative amino acid chromatography tests have given variable results. The dose of penicillamine has been kept between 2.5 and 1.5 g day. On the high dose he had slight dyspepsia ascribed to the drug. The forced fluid regimen has been continued, but the methionine-poor diet and bicarbonate medication have been discontinued. His blood pressure is normal, he has no proteinuria and the renal function is normal. Two episodes of urinary infection have been successfully treated.

Case 2

Male born 1947. A younger brother has stone-producing cystinuria, otherwise the family is healthy

When the patient was six years old, he was operated on by pyelolithotomy for the first time. In 1959 1960 and 1968 stones were removed from both renal pelves. In 1959 the main content of stone was found to be cystine. In 1968 the surgeon observed damaged renal parenchyma with dilated calyces on the right side. Between the operations multiple episodes of urinary infections, hematurias, stone-passings and transureteral removals of stones had taken place.

After the last operation in March 1968 he had slightly elevated blood pressure (160/110–155/110), no proteinuria and serum creatinine *level of 1.6–1.5 mg/100 ml*. The diagnosis of cystinuria was confirmed through positive qualitative standard tests. He had then been on methionine-poor diet and excessive fluid intake for years, and in addition bicarbonate and *d*-penicillamine 250 mg four times daily were given.

Initially the treatment was uncomplicated and *effective* apart from an ureteral obstructive calculus on the right side, which was surgically removed in Feb 1969. This stone was probably residual stone from the operation in 1968, which had diminished and passed to the ureter. *In March 1969* he observed migratory joint pains in both wrists, the small finger-joints and both knees. The pains lasted for hours and days in periods, but did not hurt much and no objective signs of joint disease were present. He was able to perform hard physical work. Small serous vesicles appeared on the dorsal side of both second fingers. At admission in May 1969 massive proteinuria of 4–9 ‰, red and white blood cells in the urine and lowered serum albumin concentration of *2.1 g/100 ml* (normal 3.2–4.1 g) were disclosed. The serum α_2-globulin fraction was slightly raised, the total protein lowered (5.6 g/100 ml) and the urine proteins mainly consisted of albumin. Serum cholesterol was elevated (435 mg/100 ml) compared with his normal level (about 220 mg/100 ml), while *renal function was still almost normal* (serum creatinine 1.4–1.3 mg/100 ml). An LE-factor test yielded strongly positive result, and LE-cells were also demonstrated.

Obviously he had developed two known sequelae to *d*-penicillamine, SLE and nephrotic syndrome. The dose

of penicillamine was first reduced to 0.5 g day and in Aug. 1969 the drug was discontinued. Renal biopsy was attempted but found technically difficult.

The joint pains subsided immediately after withdrawal of the drug, and at follow-ups in Nov. 1969 and March 1970 no laboratory signs of SLE were found. The nephrotic syndrome, however, was grossly unchanged. Therefore prednisone therapy was started in Nov. 1969 with an initial dose of 40 mg a day and successive reduction to a maintenance dose of 15 mg. In March 1970 the proteinuria was reduced to about 2 ‰, while the serum albumin was still markedly reduced, _5 g/100 ml. In May 1970 a cystine excretion of 860 mg/g creatinine was measured, an amount which usually precipitates stone formation. In Aug. 1970 the serum albumin had normalized (3.5 g/100 ml) and serum cholesterol had decreased considerably to 315 mg/100 ml. The proteinuria persisted at about 1.5 ‰. The blood pressure was 120/95 and he is subjectively healthy and fully occupied in his studies. After withdrawal of the drug he passed no stones until June 1970, when two small stones passed. X-ray showed a couple of small stones residing in the inferior calyces of the right kidney. Bicarbonate has been substituted by a trishydroxymethylaminomethane mixture, which is well tolerated and effectively keeps the urinary pH above 7.5. Serum creatinine is still almost normal (1.3–1.6 mg/100 ml).

Case 3

Female, born 1946. No member of her family had renal or inheritable disease. When six years old she had hematuria for the first time, and in the following years she suffered several small attacks of renal colics. In 1965 during her first pregnancy the attacks intensified and in March 1966 a staghorn calculus was removed by right-sided nephrolithotomy. Chemical analysis showed the stone to consist of cystine and the diagnosis was confirmed by positive cyanide-nitroprusside and amino acid chromatography tests. She was subjected to a conservative regimen with diet, fluid supply and bicarbonate, but nevertheless she passed stones repeatedly. One year after the operation, X-ray showed a new staghorn stone on the right side and several large stones in the left renal pelvis as well. Early destruction of renal parenchyma was demonstrated on the intravenous pyelogram, but serum creatinine was normal. In April 1967 *d*-penicillamine was therefore started in a dose of 500 mg 4 times daily. The effect was dramatic. A few hours after the first dose she was thrown into a series of renal colics with repeated passages of stones (Fig. 2). During the previous months she had no such attacks. After reduction of the dose the attacks ceased, but returned when a new increase was attempted. Because of the severe pains induced by this kind of stone dissolution, bilateral pyelolithotomy was performed. On operation pyramidal atrophy was observed on the right side. Following the operation penicillamine treatment was resumed in a dose of 1.5 g day.

Twenty days after the first dose of penicillamine she developed an urticarial rash with pronounced conjunctival edema. The drug was withdrawn and during the next three weeks desensitization was carried out, which was time-consuming but successful. With each increment of



Fig. 2. Case 3. A fraction of the cystine stones which passed during the first day of penicillamine treatment.

dose a slight rash and eosinophilia followed. Steroids or antihistaminics were not given. The qualitative chromatographical cystine test became negative and the cyanide-nitroprusside test remained slightly positive. She was discharged from hospital with a maintenance dose of 1.5 g *d*-penicillamine day, continued conservative regimen, iron and pyridoxine (40 mg/day) supplements.

The next six months were uneventful and without symptoms of stone formation. X-ray showed that the remaining stone on the left side had been dissolved during the period (Fig. 3). The cyanide-nitroprusside test was regularly positive. While on 1.5 g day she passed one stone; the dose was therefore increased to 2.0 g.

Despite contraceptive measures she became pregnant in Sept. 1968. This was first realized 2 months later after the most important teratogenetic period. Considering the known adverse effects of penicillamine on collagen and trace elements and the possible teratogenetic effects, both therapeutic abortion and drug discontinuation were discussed. After adequate information the patient chose to continue the pregnancy and it was also decided to continue the drug at 2.0 g day. Nevertheless she passed several stones and had renal colics almost every day during the following six months. Otherwise the course was uncomplicated. The methionine content of her diet was raised during the pregnancy.

On June 30, 1969 a girl was born. The child seemed normal apart from lax skin and slightly deformed ears. When one week old she developed signs of pyloric stenosis and operation was performed. During the following weeks several complications arose and a gross defect of connective tissue, including joint hyperflexibility, varicose veins and insufficiency of sutures, was disclosed. The child died 50 days old. Details from this course will be published elsewhere (21).

After the child-birth the patient has been well without symptoms of stone disease. X-ray in Nov. 1969 disclosed a number of small calculi in the renal parenchyma and pelvis, probably remnants of the stones produced during pregnancy. The renal function is still normal (serum creatinine 0.7 mg/100 ml) and proteinuria is not present. The cyanide-nitroprusside reaction remains slightly positive.

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CYTOLOGICAL IDENTIFICATION OF PRIMARY HEPATIC CARCINOMA CELLS

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Abstract. Cytological specimens obtained by fine-needle puncture from the liver of 3 patients with primary hepatic carcinoma and 12 patients with liver metastases of various tumours have been stained for the demonstration of naphthylamidase (N-ase) and by the MGG method. The N-ase staining method has earlier been shown to stain bile canaliculi in cytological liver preparations. In cases of primary hepatic carcinoma, bile canaliculi or canalicular fragments were recognizable among the tumour cells in preparations stained for N-ase. In two cases of bile duct carcinoma the tumour cells showed granular cytoplasmic reaction when stained for N-ase. In the remaining cases the tumour cells were N-ase-negative. Consequently staining for the demonstration of N-ase seems quite helpful when interpreting cytological liver preparations from patients suffering from neoplastic disease of the liver

Liver metastases are a common complication of malignant neoplastic disease. According to Edmondson and Anderson (2) it will be noted at autopsy that between 40 and 50% of all cancer patients have metastases within the liver In the western world primary carcinoma of the liver is found much more rarely Berman (1) has reported that primary hepatic carcinoma constitutes from 1.09 to 3.13% of all the malignant tumours discovered at autopsy in European materials.

It is usually easy to recognise metastatic tumour cells in cytological fine-needle aspirates from the liver (6, 8 11). In cases of primary liver carcinoma two types of difficulty may arise in the study of cytological smears. The malignant liver cells may be well differentiated, in cases of this nature the cytological picture resembles that of benign hepatocellular proliferation, and a histological biopsy may be needed for determination of the diagnosis. In most cases the liver cancer cells are recognised as tumour cells. However occasionally a poor degree of differentiation renders identification of the tumour cells as liver carcinoma cells a difficult task if recourse is had only to routine staining methods, such as Papanicolaou or May-Grünwald-Giemsa (MGG).

A characteristic feature of the liver cell is its ability to form bile canaliculi in the cellular membrane. In histological work primary liver carcinoma is recognisable by the occurrence of channels analogous to bile canaliculi in hepatic tumour tissue (7). The naphthylamidase (N-ase) staining method has proved very useful for the visualisation and study of bile canaliculi in cytological liver specimens (9 10 11). The purpose of the present study was to establish whether the N-ase reaction is usable for distinguishing liver cancer cells from metastatic tumour cells in cytological liver smears.

MATERIAL AND METHODS

The series comprised 15 consecutive cases of malignant liver neoplasia, each diagnosed from fine-needle aspirates. The liver biopsies were performed with Franzén instrument (3). In most cases the puncture was made intercostally in the right mid-axillary line, after locating the liver by thoracal percussion. In some cases the liver was punctured by the ventral approach, as the patient had pulmonary emphysema, or if the liver was grossly enlarged. The specimens were smeared on glass slides by the haematological technique Between four and six slides were obtained from each specimen. Half of the slides were stained for the demonstration of N-ase (9) and the other half were stained by the MGG method.

RESULTS

Table 1 shows the diagnoses, diagnostic criteria and cytochemical findings in the 15 cases. The diagnosis of primary liver cell carcinoma was confirmed at autopsy in cases _ and 3 In case 1

Table I. *Diagnosis and cytochemical findings in 15 cases of malignant liver neoplasms*

Case no.	Sex	Age (y.)	Diagnosis and diagnostic criteria	N-ase reaction of tumour cells and bile canaliculi
1	♂	62	Histol. biopsy at laparotomy: Primary hepatic carcinoma	Bile canalicular fragments among tumour cells strongly positive
2	♂	65	Histol. biopsy at laparoscopy: Cirrhosis. Autopsy: Primary hepatic carcinoma	Bile canalicular fragment among tumour cells strongly positive
3	♂	68	Autopsy: Primary hepatic carcinoma	Bile canaliculi surrounding tumour cells strongly positive, canalicular pattern disorganized
4	♂	52	Histol. biopsy at laparotomy: Pancreatic carcinoma + liver metast.	Negative
5	♀	73	X-ray: Hypernephroma + pulmonary metast.	Negative
6	♀	45	Clinical: Liver metast. of operated mammary carcinoma	Negative
7	♀	83	Autopsy: Carcinoma of gall bladder + liver metast.	Negative
8	♀	59	Autopsy: Carcinoma of pancreas + liver metast.	Negative
9	♂	61	Histol. biopsy: Pulmonary carcinoma + liver metast.	Negative
10	♂	49	Autopsy: Bronchial carcinoma + liver metast.	Negative
11	♂	68	Autopsy: Pulmonary carcinoma + liver metast.	Negative
12	♀	54	Clinical: Liver metast. of operated mammary carcinoma	Negative
13	♂	64	Autopsy: Carcinoma of pancreas + liver metast.	Negative
14	♀	70	Autopsy: Carcinoma of bile ducts	Granular reaction in cytoplasm
15		80	Autopsy: Carcinoma of bile ducts	Granular reaction in cytoplasm

the diagnosis was confirmed histologically during laparotomy. In case 2 a laparoscopy was performed shortly after the first fine-needle biopsy had been obtained. A histological preparation of a liver specimen obtained during laparoscopy revealed changes typical of hepatic cirrhosis, whereas no signs of malignancy were detectable. Furthermore, the visual impression of the liver surface conduced to the diagnosis of cirrhosis. Some time after the laparoscopy two further fine-needle punctures were performed in view of the unexpected histological diagnosis. The impression of all three fine-needle biopsies was that of primary hepatic carcinoma.

Liver neoplasm other than primary carcinoma was the diagnosis in the 12 remaining cases. The occurrence of liver metastases was demonstrated at autopsy in seven and by histological biopsy in two cases. In one case the metastatic nature of the liver tumour was strongly suggested by changes typical of primary renal cancer as revealed by an intravenous pyelography. Two patients had undergone surgery for mammary carcinoma 2 and 5 years before the onset of liver symptoms; consequently the diagnosis of metastatic hepatic neoplasm seemed highly probable.

Tumour cells were easily recognised in MGG-stained preparations of patients 1–2 (Fig. 3), and 4–15 (Fig. 7). In patient 3 the tumour cells were rather well differentiated (Figs. 5–6): a proliferative process was indicated by nucleolar enlargement and marked anisokaryosis, which were the only pathological features. In other respects the cells resembled normal liver cells. A definite cytological diagnosis was not possible, but hepatocellular malignancy was suspected.

In cases 1–2 staining for the demonstration of N-ase revealed the occurrence of fragments of enzyme-positive bile canaliculi among the tumour

Fig. 1. Normal liver cells with pigment granules and a few fat vacuoles. MGG, ×260. *Fig. 2.* Normal liver cells with regular bile canaliculi. N-ase, ×260. *Fig. 3.* Primary liver cancer (case 2). MGG ×660. *Fig. 4.* Primary liver cancer (case 2). N-ase, ×140. *Fig. 5.* Primary liver cancer (case 3). MGG, ×660. *Fig. 6.* Primary liver cancer (case 3). N-ase, ×260. *Fig. 7.* Metastatic tumour cells in the liver (case 9). MGG ×260. *Fig. 8.* Metastatic tumour cells in the liver (case 9). N-ase, ×260.

cells (Fig. 4). Some degree of diffuse cytoplasmic N-ase activity was observable in part of the tumour cells. The enzyme-positive groups of tumour cells were easily distinguishable from normal hepatocytes by the complete irregularity and fragmentation of the bile canaliculi.

In cases 4–13 the tumour cells were completely devoid of N-ase activity (Fig. 8). In cases 14 and 15 they revealed a granular enzyme-positive reaction. Nevertheless bile canaliculi or canalicular fragments could not be observed among the tumour cells.

DISCUSSION

The fine-needle aspiration of cytological material from the liver is a safe and technically simple procedure. A cytological diagnosis is practicable in many cases of hepatitis, cholestasis, steatosis, and haemosiderosis (4, 5, 8, 11), although the cytological study cannot replace histological examination in all cases. For unequivocal diagnosis of cirrhosis of the liver histological specimens are usually required, notwithstanding the risks connected with the puncture. For the diagnosis of metastatic tumours of the liver the fine-needle method is superior. In view of the negligible risk cells may be aspirated from large areas of the liver and the chance of hitting tumour tissue is improved.

To overcome the difficulties that arise in the interpretation of cytological material from patients with primary hepatocellular cancer, the N-ase staining method for the demonstration of bile canaliculi seems quite helpful. In cases of anaplastic liver cancer the presence of canalicular structures in the groups of tumour cells must be considered definite proof of the hepatocellular origin of the tumour. We have never found any structures which resemble bile canaliculi in metastatic tumour tissue of the liver. On the other hand, if the tumour cells are better differentiated and are identified as liver cells, the irregular and bizarre canalicular pattern found in conjunction with liver cancer might be considered a sign of malignancy.

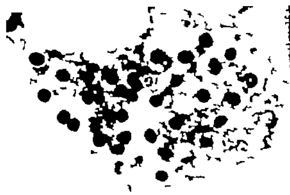
Bile duct endothelium cells are recognisable by their typical granular staining properties in preparations stained for the demonstration of N-ase. This also seems to be the case with carcinoma cells originating from the bile ducts (cases 14–15). It seems, however, that cancer cells from the distal parts of the bile duct tree, at least the gall bladder (case 7), are N-ase-negative. These cells cannot be distinguished from other types of metastatic carcinoma cells of the liver by application of the N-ase reaction.

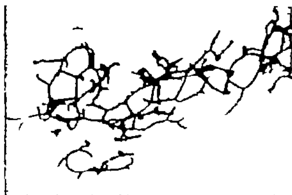
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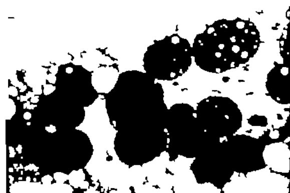
This study was supported by a grant from the Sigrid Jusélius Foundation.

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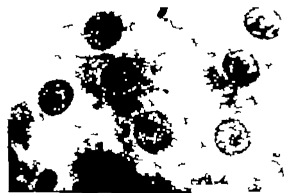
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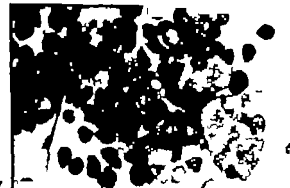

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followed by rapid hemolysis, thrombocytopenia and progressive deterioration of renal function, unresponsive to high-dose therapy with prednisone and to splenectomy The significance of the renal transplant as a target organ is evidenced by the immediate disappearance of thrombocytopenia after removal of the graft. It seems reasonable to assume that thrombocytopenia was due to consumption of platelets in the renal arteriolar and glomerular thrombi, a process which may have included the participation of complement.

In patients with hemolytic-uremic syndrome who die from renal failure several kinds of renal lesions occur cortical necrosis, glomerulonephritis and vascular lesions in arterioles and capillaries with the presence of multiple thrombi as in thrombotic, thrombocytopenic purpura.

The renal lesions in the present case were predominantly or exclusively of vascular origin, i.e. small vessel thrombi and arteriolar thickening and fibrinoid necrosis. Only mild arterial intimal thickening such as occurs in chronic allograft reaction was seen in the present case· such an event, however seems unlikely in view of the complete lack of clinical evidence for this condition, until the acute onset of hemolysis and rapidly deteriorating graft function. Histological signs of acute cellular allograft reaction were totally absent, and immune deposits could not be demonstrated in the glomerular capillary walls.

The term microangiopathic hemolytic anemia has been introduced to designate the presence of hemolytic anemia with red cell fragmentation and vascular lesions in various disease states, including the hemolytic-uremic syndrome. The subject has been reviewed recently by Bram (2). The clinical features as well as experimental models suggest that hemolysis might result from direct contact between the patient's red cells and the vascular lesions. The notion that thrombocytopenia is brought about by removal of platelets from the circulation by thrombus formation and contact with the renal microvascular disease is strongly supported by the effect of graft-nephrectomy in the present case. Microangiopathic hemolytic anemia has been reported in association with acute rejection of a liver transplant (3) and in three renal allograft recipients (8, 9) within two months after transplantation. As mentioned above, we consider rejection unlikely as initiating event in the present case.

The etiology and pathogenesis of hemolytic uremic syndrome remains unknown. Evidence of a viral agent was presented by Gianantonio et al. (5), who found serologic conversion in two-thirds of 54 patients and also claimed to have isolated an Arbovirus from the blood in 8 patients. Glasgow and Balduzzi (6) isolated a Coxackie virus in one patient, and in our case seroconvention to influenza A virus was found. If as suggested by these findings, a virus infection could trigger an (immunological) process leading to this syndrome, it would appear that allotransplant recipients may incur a particular risk due to immunosuppressive therapy The increased frequency of several virus infections in these patients is well documented (1 10)

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Acta med. scand. Vol. 189 pp. 381–384 1971

INFLUENCE OF THE HYPOTHYROID STATE ON LIPOLYSIS IN HUMAN ADIPOSE TISSUE IN VITRO

Hypothyroid State and Lipolysis

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Abstract. The regulation of lipolysis has been studied in subcutaneous adipose tissue removed under local anaesthesia from hypothyroid patients. As control subjects were used hypothyroid subjects on replacement therapy. Noradrenaline induced no significant increase in lipolysis in tissue removed from hypothyroid patients, while the increase in glycerol release from adipose tissue of the control group was almost twofold. The addition of the alpha-adrenergic blocking agent phentolamine to noradrenaline-containing media produced significant increase in the glycerol release from tissue of the hypothyroid patients as well as from the control subjects. Isopropylnoradrenaline, nearly selective beta-adrenergic agonist, theophylline or dibutyryl-cyclic AMP stimulated lipolysis in tissue from both groups. These results indicate that the lipase system cannot be the rate-limiting factor for the lipolytic response to noradrenaline in adipose tissue from hypothyroid patients. The decreased lipolytic response to noradrenaline in this tissue seems to be due to more pronounced alpha-adrenergic effect of noradrenaline counteracting the lipolytic effect mediated by the beta-adrenergic receptor.

It is well established that thyroid hormones play an important role in lipid mobilisation (17). Increased lipolysis in hyperthyroid patients has been demonstrated by measuring both plasma glycerol concentration and turnover (16). Furthermore, Harlan et al. (9) showed an enhanced lipolytic response to adrenaline and noradrenaline in hyperthyroid and a reduced response in hypothyroid patients by measuring the increase in plasma FFA to intravenous infusion of the two catecholamines.

In order to further investigate the regulation of lipolysis in adipose tissue from hypothyroid subjects we have studied subcutaneous adipose tissue specimens in vitro. The main finding was that noradrenaline, a potent lipolytic agent in human adipose tissue in vitro, had no effect on adipose tissue from hypothyroid subjects (7, 8, 13, 19). The data indicate that this result was due to an increased alpha-adrenergic receptor response to noradrenaline.

MATERIAL AND METHODS

The 11 female subjects included in this study had developed hypothyroidism after radioiodine treatment for thyrotoxicosis. The diagnosis was established on the basis of clinical examination and the available laboratory investigations. Clinical data and the results of the laboratory tests on the subjects are given in Table I.

The five controls were hypothyroid subjects on replacement therapy with thyroid hormone (desiccated thyroid, 75 mg, Thyranon® or *l*-thyroxine, 0.1 mg, Levaxin®). The patients in the control group had been on this therapy and followed for seven months up to nine years. None of them were over-substituted. The replacement dose was probably low in two subjects. In one (no. 1), it was deliberately kept low because of the patient's cardiosclerosis. In the other subject (no. 5) it could not be further increased due to the occurrence of palpitations with higher dose of thyroid hormone. In two subjects (nos. 3 and 6) high PBI values were caused by iodine-containing medicine for conditions not connected with thyroid disease.

Subcutaneous adipose tissue was excised from the thigh under local anaesthesia using 3–5 ml of 0.5% prilocain chloride, Citanest®. The subjects were ambulant and non-fasting. The fat tissue specimens were transported in 0.9% NaCl at 37°C and preincubated for one hour at 37°C in Krebs-Henseleit bicarbonate (KHB) buffer containing 1% bovine albumin. Separate tissue sections (50–100 mg) were then incubated in 1.5 ml of KHB (pH 7.4) containing 3% bovine albumin (Fraction V, Armour Pharmaceutical Division, lot number MG

Table I. *Clinical and laboratory findings in the subjects studied*

Pat. no.	Age (y)	Sex	PBI (μg/100 ml)	Cholesterol (mg/100 ml)	Resin uptake of T_3-¹²⁵I (%)	Thyroidal 24-h uptake f ¹³¹I (%)	Treatment Dose mg/d	Duration
Control group								
1	44	♀	4.6	315	30	—	Thyranon 113	9 y
2	50	♀	4.8	271	25	—	Thyranon 75	6 y
3	58	♀	30.0[a]	331	28	—	Levaxin 0.2	7 mo
4	64	♀	5 0	268	—	—	Thyranon 75	9 y
5	66	♀	3.9	324	31	—	Thyranon 56	9 y
Hypothyroid group								
6	48	♀	9 7[a]	536	21	1	—	
7	52	♀	3.2	406	24	28	—	
8	54	♀	2 3	463	25	—	—	
9	56	♀	2.7	375	27	20	—	
10	62	♀	3.0	376	27	17	—	
11	69	♀	3.4	341	24	20	—	

[a] Abnormal value due to iodine def ciency.

2770), providing pproximately 0.35 μmoles f FFA/ml of medium, and 1 mg/ml of glucose.

The incubations were carried out fo two hours in polyethylene vials (Packard Co., La Grange Ill.) at 37°C using air as gas phase.

All lipolytic agents as well as the basal glycerol release were tested on each subject in duplicate incubations.

Aliquots of the medium were removed at the end of the incubation, and glycerol was determined according to Wieland (18) as modified by Larsen (10). I prior experiments, Östman et al. (19) have shown that the production of glycerol is linear with time in normal human adipose tissue under the conditions used.

In each experiment the lipolytic effect of the following agents was tested *l*-noradrenaline bitartrate (Astra Läkemedel AB, Sweden), *l*-isopropylnoradrenaline-*d* bitartrate dihydrate (Dr F P Ludueña, Sterling-Winthrop Research Institute), phentholamine, Regitine® (Ciba), N⁶ 2'O-dibutyryl-3',5'-monophosphate (Boehringer/Mannheim, West Germany). Theophylline was obtained commercially. *Catecholamines and theophylline* were used at concentrations known to produce maximal stimulation in subcutaneous adipose tissue from normal subjects (7). I separate experiments on human adipose tissue it was established that the local anesthetic agent prilocain chloride had no ffect on glycerol release induced by the lipolytic agents under identical conditions to those used in the present study (Arner & Östman, in preparation)

PBI was determined by the auto-analyser technique by Riley and Gochman (14) (normal range 4–8 μg/100 ml).

Cholesterol was measured according to Pearson et al. (13) (normal range 150–300 mg/100 ml).

For the ¹²⁵I-triiodothyronine resin sponge test the method described by Bois-Svensson et al. (3) was used (normal range 25–35%).

The radioiodine uptake in the thyroid gland was determined at 24 hours in accordance with the recommendations drawn up at a consultants meeting in 1960 convened by the International Atomic Energy Agency (5).

RESULTS

The basal glycerol release was nearly the same in tissues obtained from the control and the hypothyroid subjects (Table II) The addition of nor adrenaline at a concentration of 3×10^{-6} M significantly ($p < 0.02$) increased the glycerol release in specimens from control subjects but did not stimulate lipolysis in adipose tissue from the hypothyroid patients. However a nearly pure beta-adrenergic agonist, *l*-isopropylnoradrenaline (3×10^{-6} M) induced a significant lipolytic response in tissues from control as well as from the hypothyroid subjects. Theophylline (10^{-3} M) and the dibutyryl derivate of cyclic AMP (10^{-3} M) markedly stimulated lipolysis in both kinds of adipose tissue.

By addition of 0.5 μg/ml of alpha-adrenergic blocking agent, phentholamine, to noradrenaline-containing media a significant increase in lipolysis was observed in tissue from the control as well as from the hypothyroid subjects.

DISCUSSION

It is known that lipolysis in adipose tissue from the rat (4) and man (6, 7) is controlled by cyclic adenosine 3',5'-monophosphate (c-AMP) which activates the hormonal sensitive lipase. The lipolytic effect of catecholamines is mediated by the adrenergic receptors and adenyl cyclase which converts ATP to c-AMP (4 15).

Two types of adrenergic receptors have been

Table II. *Effect of lipolytic agents and an alpha-adrenergic blocking compound on the glycerol release from subcutaneous adipose tissue from euthyroid and hypothyroid subjects*

Addition to medium	Control (= 5)			Hypothyroid ($n = 6$)		
	Mean ±S.E.M.	Mean diff.[b] ±S.E.M.	p	Mean ±S.E.M.	Mean diff.[b] ±S.E.M.	p
None	0.86±0.15			1.04±0.18		
l-noradrenaline 3 10^{-6} M	1.67±0.13	0.81±0.21	<0.02	1.03±0.15	−0.02±0.28	N.S.
l-isopropylnoradrenaline 3 10^{-6} M	3.28±0.13	2.41±0.27	<0.001	[illegible].59±0.47	1.55±0.41	<0.02
Dibutyryl -AMP 10^{-3} M	3.48±0.23	2.62±0.32	<0.005	2.77±0.15	1.73±0.16	<0.001
Theophylline 10^{-3} M	3.10±0.38	2.24±0.40	<0.01	3.09±0.33	2.05±0.24	<0.001
l-noradrenaline 3 10^{-6} M + phentholamine 0.5 µg/ml	3.04±0.24	2.18±0.22	<0.001	2.26±0.43	1.22±0.42	<0.05

[a] µmoles/g wet weight per two hours of incubation. Mean ± standard error

[b] The significance was tested from the paired difference between glycerol release in presence and absence of the different additions to the medium indicated in the Table.

demonstrated by Ahlquist (1), the so called alpha and the beta-adrenergic receptors. Both exist in human adipose tissue where the beta-adrenergic type mediates the lipolytic effects of catecholamines while the alpha-adrenergic receptors inhibit lipolysis (7). Thus the lipolytic activity of noradrenaline, which stimulates both types of receptors, will depend on the relative influence of the two on lipolysis. Isopropylnoradrenaline, on the other hand, which mainly acts on the beta-adrenergic receptor should be lipolytic even if the responsiveness of the alpha-adrenergic receptor is increased. Two other agents were used to study the lipolytic mechanisms: theophylline, which blocks the degradation of c AMP and the dibutyryl derivate of c AMP which, unlike c AMP easily penetrates cell membranes and activates the hormone sensitive lipase (2).

In the present in vitro study the basal lipolysis was not significantly different in the two groups. This is in accordance with the observation of Laurell and Tibbling (11), who found that the glycerol concentration in plasma of hypothyroid is not significantly different from that of euthyroid subjects.

The lipolytic response to noradrenaline was absent in subcutaneous adipose tissue from hypothyroid subjects, whereas isopropylnoradrenaline, theophylline and dibutyryl c-AMP had a potent lipolytic effect in parallel incubations. The net glycerol release induced by these agents was not significantly higher in the control group although the mean values were always numerically greater. Hence, the lack of lipolytic effect of noradrenaline on tissues from hypothyroid subjects cannot be due to a decreased lipase content. The finding that isopropylnoradrenaline alone, as well as noradrenaline in the presence of an alpha-adrenergic antagonist, significantly stimulated lipolysis in adipose tissue from hypothyroid patients sheds light on the mechanism behind the lowered noradrenaline response in this tissue. The beta-adrenergic receptor must be functioning in order to respond to isopropylnoradrenaline or noradrenaline in the presence of phentholamine. However when both the alpha and the beta-adrenergic receptors were activated by noradrenaline alone, no increase in lipolysis was observed in the tissue from the hypothyroid subjects, indicating that there must be an enhanced alpha-adrenergic receptor response to noradrenaline which counteracts the lipolytic effect mediated by the beta adrenergic receptor.

These studies thus indicate that thyroid hormones may play an important role in controlling the balance between the adrenergic receptors, the hypothyroid state favouring an increase of alpha-adrenergic receptor potency. This analysis assumes that phentholamine acts as an alpha-adrenergic antagonist. However it does not exclude other possible explanations of the observed unresponsiveness to noradrenaline such as changed tissue metabolism of norepinephrine in the hypothyroid state (17). Investigations are in progress in this laboratory in order to elucidate whether an alpha receptor enhancement can be demon-

strated also in vivo in adipose and other tissues in patients with hypothyroidism.

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Acta med. scand. Vol. 189 pp. 385–389 1971

PLATELET FUNCTION AND PLATELET PHOSPHOLIPIDS IN PATIENTS WITH HYPERBETALIPOPROTEINEMIA

Effect of Nicotinic Acid and Clofibrate

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Abstract. Platelet factor-3 activity and availability platelet phospholipids, their fatty acid and aldehyde composition and plasma lipids have been examined in patients with hyperbetalipoproteinemia before and after treatment with nicotinic acid or clofibrate. Increased platelet factor 3 activity was present in platelet-rich plasma, and increased availability was found after exposure of platelets to adenosine diphosphate. Estimated per 10^9 platelets, increased amounts of platelet phospholipids were found in the patients, whereas the phospholipid fatty acid pattern was as in normals. After treatment with nicotinic acid and also clofibrate, the platelet factor 3 activity and availability were normalized. No changes were observed in platelet factor 3 activity in platelet-poor plasma or in the quantity of platelet phospholipids and their fatty acids during the treatment. Plasma cholesterol and phospholipids were markedly decreased by the treatment. It is suggested that an interaction exists between platelets and plasma lipoproteins. In patients with hyperbetalipoproteinemia this interaction may favour the tendency to thrombosis and can be reversed by treatment with nicotinic acid and probably clofibrate.

Patients with hyperbetalipoproteinemia (type II) have a high frequency of occlusive arterial diseases, especially involving the coronary arteries (6). This tendency has mainly been associated with the high plasma cholesterol levels. The significance of increased plasma cholesterol as a risk factor in the development of ischemic heart disease is well established from larger groups of subjects (8, 19).

Thrombus formation has a fundamental role in the pathogenesis of occlusive arterial disease. Thrombosis often represents the final occluding event in atherosclerotic arteries, and there is good evidence for the transformation of mural thrombi into atherosclerotic lesions (9). During thrombus formation a series of events take place, including adhesion and aggregation of platelets, release of platelet nucleotides and exposure of coagulation active phospholipids on the platelet surface (10). These platelet lipids participate in the coagulation process, eventually leading to fibrin formation and consolidation of the thrombus.

In the present study we have examined the activity and availability of platelet factor 3 and the biochemical nature of the platelet phospholipids in patients with familial hyperbetalipoproteinemia and in normals. In a subgroup of patients we have also examined the platelets after treatment with the cholesterol lowering agents nicotinic acid and clofibrate. The plasma lipids were also included in the study.

MATERIAL

Venous blood was collected after 12–14 hours fasting. Medical and dietary history, physical and hematologic examinations including estimation of the main plasma lipid fractions, were carried out. The control group comprised 20 healthy male subjects between 22 and 63 years of age. The patient group comprised 12 subjects, 4 females and 8 males between 20 and 70 years of age. Tendinous xanthoma was present in 9 cases, xanthelasma in 8 and corneal arcus in all of them. Four patients had symptoms of ischemic heart disease with electrocardiographic abnormalities. The family history gave information of the occurrence of xanthoma, xanthelasma or ischemic heart disease in all patients. All patients were on an ordinary Norwegian diet and no dietary changes were made during the experimental period. Nicotinic acid and clofibrate were the only drugs used during the study.

Seven patients were treated with nicotinic acid, 3 g daily for 12 weeks, and then continued immediately on a daily dose of [illegible] g clofibrate for another 12 weeks. The clofibrate used was Atromid-S (Imperial Chemical Industries Ltd.).

Table I. *Serum lipids in patients with hyperlipoproteinemia (type II) and in a control group*

	Normal ($n = 17$)	Type II ($n = 12$)
Age (y)	39 (22–63)	49 (28–70)
Total cholesterol (mg/100 ml)	260 ± 50	486 ± 157[a]
Triglycerides (mg/100 ml)	78 ± 49	101 ± 36
Phospholipids (mg/100 ml)	214 ± 25	378 ± 89[a]
Unesterified fatty acids (μEq/l)	594 ± 120	555 ± 180

[a] $p < 0.01$.

Table II. *Stypven time (PF 3 activity) in platelet-rich and platelet-poor plasma from normals and patients with type II hyperlipoproteinemia*

No of platelets (mm³)	Test reagent	Stypven time (sec) Normal ($n = 27$)	Type II ($n = 8$)
300 000	Buffered saline	43.0 ± 4.0 (34.7–56.6)	35.3 ± 2.4[a] (32.3–35.6)
300 000	Kaolin (1 mg/ml)	28.8 ± 3.8 (21.8–36.6)	28.4 ± 3.9 (24.7–33.4)
300 000	ADP (1 × 10⁻⁶ M)	34.4 ± 3.2 (25.5–43.9)	31.7 ± 3.3[a] (25.9–36.5)
300 000	Frozen and thawed three times	15.2 ± 1.6 (12.7–19.0)	15.3 ± 1.1 (14.0–16.7)
15 000	Buffered saline	59.5 ± 8.0 (44.4–84.1)	55.0 ± 3.5[b] (48.4–67.4)

[a] $p < 0.01$
[b] $0.01 < p < 0.05$.

METHODS

The serum lipoproteins were separated by the method of Noble (14) using agar-agarose electrophoresis. The serum levels of total cholesterol, triglycerides, phospholipids and unesterified fatty acids were measured as earlier described (17).

The platelet studies, including estimation of platelet factor 3 (PF-3) activity and availability after exposure of platelet-rich plasma to kaolin and ADP fractionation of phospholipids with estimation of the phospholipid fatty acids and aldehydes on thin layer and gas liquid chromatography and protein estimation, have recently been described in detail (15, 17).

RESULTS

All patients were in good condition, with no signs of acute disease at the time of the investigation. The diagnosis was confirmed by serum lipoprotein electrophoresis. All patients had a hyperbetalipoproteinemia (type II) with high levels of total cholesterol and phospholipids in serum, whereas the triglyceride level was within the normal range, as shown in Table I.

Compared with the control group, increased platelet factor 3 activity was found in platelet rich plasma from the patients. Following exposure to ADP the patients platelets also showed increased availability of PF-3 as shown in Table II. The "total" activity of PF-3 measured in platelet rich plasma frozen and thawed three times did not differ in the two groups. This may be due to the low sensitivity of the method at short clotting times.

The total amount of platelet phospholipids was moderately increased in the patients (Table III). However the percentage distribution of the main phospholipid fractions and the phospholipid/protein ratio were similar in the two groups (Table IV). The increase of the total platelet phospholipids reflected a general, though slight, increase of all phospholipid fractions. The fatty acid and aldehyde pattern of the phospholipids showed no

Table III. *Phospholipid composition of platelets from normals and patients with type II hyperlipoproteinemia*

Compound	Normal ($n = 27$) (μg lipid P/10⁹ platelets)	%	Type II ($n = 12$) (μg lipid P/10⁹ platelets)	%
P E.	3.10 ± 0.30	31.6	3.40 ± 0.70	29.9
P S.	0.66 ± 0.10	6.8	0.91 ± 0.23	8.4
P L	0.29 ± 0.06	3.0	0.30 ± 0.10	2.6
P G	4.39 ± 0.30	44.8	4.81 ± 0.96	43.6
Sph.	1.35 ± 0.16	13.8	1.52 ± 0.38	13.8
Total recovered lipid *P*	9.79 ± 0.80		11.00 ± 1.90[a]	

[a] $p < 0.01$

Table IV. *The phospholipid/protein ratio in platelets from normals and patients with type II hyperlipoproteinemia*

	Normal ($n = 17$)	Type II ($n = 12$)
Phospholipid (mg) / Protein (mg)	0.14 ± 0.02	0.15 ± 0.02

Table V. *Fatty acid distribution (g/100 g of fatty acids and aldehydes) of ethanolamine phosphoglycerides and serine phosphoglycerides in platelets*

Components	Ethanolamine phosphoglycerides		Serine phosphoglycerides	
	Normal (n = 17)	Type II (n = 9)	Normal (n = 17)	Type II (n = 9)
16:0 DMA	6.1±1.6	5.6±2.6		
16:0	6.9±1.4	5.0±2.4	8.8±5.0	4.5±1.3
16:1	0.5±0.1	0.6±0.2	1.1±0.2	0.5±0.2
17:0	0.4±0.1	0.2±0.1	0.2±0.1	0.2±0.1
18:0 DMA	8.4±1.8	9.2±2.8		
18:0, 18:1 DMA	22.5±4.5	24.2±8.3	44.2±8.0	47.8±6.5
18:1	11.0±2.0	11.2±3.5	22.8±3.1	25.5±2.6
18:2	3.4±1.0	2.8±1.0	2.2±0.6	1.7±0.4
20:0	0.5±0.1	0.8±0.2	1.4±0.2	1.6±0.4
20:1, 18:3	1.0±0.2	1.0±0.3	1.0±0.1	1.5±0.4
20:3, 22:0	1.3±0.4	0.8±0.2	1.8±0.3	2.0±0.7
20:4, 22:1	25.6±6.0	28.3±6.7	14.0±3.5	14.7±5.2
20:5, 22:2	1.5±0.3	2.1±0.4	0.9±0.1	0.4±0.1
22:4	2.0±0.8	1.4±0.4	1.0±0.1	0.6±0.2
22:un	2.8±0.5	2.8±0.5		
22:5	2.9±0.8	2.8±0.6		
22:6	3.9±1.0	2.5±0.8		
26:1	Trace			

Table VI. *Fatty acid distribution (g/100 g of fatty acids and aldehydes) of choline phosphoglycerides and sphingomyeline of platelets*

Component	Choline phosphoglycerides		Sphingomyeline	
	Normal (n = 17)	Type II (n = 10)	Normal (n = 16)	Type II (n = 8)
16:0	34.5±3.5	33.6±4.0	20.3±7.2	20.9±1.9
16:1	1.5±0.2	1.6±0.3	0.3±0.1	0.9±0.4
17:0	0.5±0.1	0.4±0.1	1.8±0.4	0.3±0.1
18:0	16.2±1.0	15.1±2.4	9.9±5.0	5.5±1.5
18:1	24.0±3.1	26.6±2.9	6.3±2.8	5.9±4.0
18:2	7.4±1.7	7.8±1.3	0.3±0.1	0.3±0.1
20:0	1.0±0.2	1.3±0.3	4.3±1.6	6.5±2.4
20:1, 18:3	2.1±0.4	2.8±0.5		
20:3, 22:0	1.5±0.2	1.5±0.4	20.6±6.0[a]	21.9±5.8
20:4, 22:1	10.5±4.8	9.4±3.9	13.2±3.1[b]	8.6±1.5
20:5, 22:2	0.5±0.1	0.3±0.1		
22:4, 23:0	0.4±0.1	0.2±0.1	1.0±0.4	1.5±0.6
24:0			8.8±2.4	11.5±4.8
24:1			14.6±2.1	15.2±4.0
22	0.4–0.2	Trace		
22:5	1.0–0.4	Trace		

[a] Appears to be largely 22:0.
[b] Appears to be largely 22:1.

Table VII. *Percentage composition of fatty chains of platelet phospholipids in normals and in patients with hyperlipoproteinemia (type II)*

Compound	Saturated fatty acids + aldehydes/unsaturated fatty acids	
	Normal (n = 17)	Type II (n = 12)
P.E.	0.82	0.82
P.S.	1.18	1.17
P.C.	1.08	1.02
Sph.	2.00	2.12

Table VIII. *Serum lipids in seven patients with hyperlipoproteinemia (type II) before and after treatment with nicotinic acid or clofibrate*

	Before treatment	Nicotinic acid (3 g/d. for 3 mo.)	Clofibrate (2 g/d. for 3 mo.)
Total cholesterol (mg/100 ml)	534±186	451±208[a]	405±169[a]
Triglycerides (mg/100 ml)	104± 32	90± 24	101± 31
Phospholipids (mg/100 ml)	378± 89	282± 59[a]	275± 83[a]

[a] $p < 0.01$

significant differences between the patients and the controls (Tables V, VI and VII).

The effect of nicotinic acid and clofibrate

A group of seven patients were treated with nicotinic acid for three months, followed by a three months period on clofibrate. A significant reduction of serum cholesterol and phospholipids was obtained with nicotinic acid (Table VIII). The reduced lipid levels were maintained by the clofibrate medication.

The high platelet factor 3 activity present in platelet-rich plasma from patients with hyperlipoproteinemia was significantly reduced during treatment both with nicotinic acid and clofibrate (Table IX). PF 3 availability after exposure of PRP to ADP was also reduced after treatment with nicotinic acid, whereas PF 3 activity in PPP was unaffected by the treatment. Thus, the PF-3 activity showed normalization during treatment, particularly with nicotinic acid.

No significant biochemical changes in platelet phospholipids or in their fatty acid pattern were observed during the treatment periods.

Table IX. *Stypven time (PF 3 activity) in platelet rich and platelet poor plasma from 7 patients with hyperlipoproteinemia (type II) before and after treatment with nicotinic acid or clofibrate*

No. of platelets/mm³	Test reagent	Before	Nicotinic acid (3 g/d. for 3 mo.)	Clofibrate (2 g/d. for 3 mo.)
300 000	Buffered saline	35.4±2.4	43.5±1.8[a]	41.2±3.0[a]
300 000	Kaolin (1 mg/ml)	28.6±4.6	29.6±1.8	28.0±1.8
300 000	ADP (1 × 10^{-4} M)	31.4±4.0	34.9±2.8[b]	33.0±4.0
300 000	Frozen and thawed three times	15.3±1.0	15.8±1.0	15.4±1.1
15 000	Buffered saline	55.1±3.6	54.1±4.3	58.3±2.3

[a] $p < 0.01$
[b] $0.01 < p < 0.05$.

DISCUSSION

The present study has shown that patients with hyperbetalipoproteinemia in addition to severe abnormalities in plasma lipids have changes in their platelet function. Platelets provide coagulant activity (PF 3) to the plasma coagulation process. This activity is closely related to the platelet phospholipids. When platelets are exposed to a series of physiological substances or mechanical strain, this activity is made available. The patient group showed consistently increased PF 3 activity in platelet-rich plasma, and they also had increased availability of PF 3 activity after exposure of platelets to adenosine diphosphate, which seems to be the mediator for most platelet aggregating substances.

The mechanisms involved in the registered platelet factor-3 activity are not known. The present study has shown increased amounts of total platelet phospholipids in these patients, a pattern earlier described in patients with ischemic heart disease (16). This may account for the increased PF 3 activity and could reflect an increased platelet turnover with a young platelet population (12). As the fatty acid pattern of the phospholipids was similar to that found in the control group, it is unlikely that the fatty acids are responsible for the differences in activity.

Earlier studies have shown that betalipoproteins added to human platelet-rich plasma accelerates ADP and thrombin-induced platelet aggregation (5). Increased platelet aggregation has also been observed in patients with essential hyperlipemia with high triglyceride levels (18). These observations indicate a connection between the plasma lipoproteins and platelet function. The increased platelet factor 3 activity in patients with hyperbetalipoproteinemia may therefore reflect changes on the platelet membrane induced by the plasma lipoproteins.

When the patients were treated with nicotinic acid, a significant reduction in plasma cholesterol and phospholipids was observed. This is followed by a concomitant reduction of betalipoproteins. The platelet factor 3 activity was simultaneously reduced in PRP whereas it remained unchanged in PPP. No significant changes were observed in the quantity of the platelet phospholipids (19), and the relative composition of the platelet phospholipid fatty acids did not change during clofibrate treatment, in opposition to what has been reported in serum phospholipid fatty acids and red cell free fatty acids (3, 11).

The present observations thus strongly indicate an interaction between plasma lipoproteins and platelets. The changes in PF 3 activity were not observed in platelet-poor plasma, indicating that the test system was not influenced by the changes in plasma lipids and lipoproteins alone. The changes in platelet-rich plasma indicate that a reduction of betalipoproteins, probably on the platelet surface, may be responsible for the reduced platelet factor 3 activity in patients treated with nicotinic acid or clofibrate.

Earlier studies have shown that clofibrate reduces platelet stickiness and decreases platelet turnover (2, 7), though more recent studies seem to indicate that the influence on platelet stickiness is transient (4). As regards nicotinic acid, recent studies have shown reduced platelet factor 3 activity shortly after administration of this drug (13). This report could indicate that the plasma level of free fatty acids also influences the platelet factor 3 activity (1).

On the basis of the present study it is suggested that platelets in patients with hyperbetalipoproteinemia are affected by the plasma lipoproteins. This interaction could increase the tendency to thrombosis and could be counteracted by nicotinic acid and probably clofibrate.

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PREDICTIVE CRITERIA OF SURGICAL CURABILITY OF RENOVASCULAR HYPERTENSION

Comparative Assessment Individually and in Combination by Discriminant Analysis

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Abstract. The data available before the hypertensive patient undergoes surgical treatment for renovascular hypertension have been assessed as to their value in predicting the effect of the operation on the hypertension. The 43 patients studied were selected to exclude secondary complications and technically unsatisfactory surgical procedures. The data were assessed individually and also by discriminant analysis to determine the best combination of criteria for preoperative prediction of surgical results. The best individual criteria were (in order of decreasing importance): 1) increased urinary concentration of creatinine or urea on the diseased side or increased plasma renin activity (PRA) in the suprarenal inferior vena cava; 2) hemodynamic delay beyond the arterial stenosis (renal arteriogram); 3) duration of hypertension, and 4) sum of criteria from excretory urogram. Combination of clinical and roentgenologic criteria provided correct prediction in 80 to 85% of the cases, which is about the accuracy provided by the best individual criteria.

The major concern raised by the discovery of a renal artery lesion on the arteriogram of a hypertensive patient is to determine whether or not surgical treatment will produce cure or amelioration of the hypertension. Two types of evidence have shown that the coexistence of a renal artery lesion and hypertension does not necessarily imply a causal relationship: the inconsistent results of nephrectomy or of the surgical repair of the renal artery lesion (10–33–34) and the presence of renal artery stenosis in normotensive subjects (9, 11, 17, 41).

There are numerous reports assessing the value of the criteria of surgical curability of hypertension associated with renovascular lesions. However, these studies were based on small groups of patients, not always comparable, the criteria were assessed individually and the conclusions were frequently contradictory.

Uncertainty remains especially as regards clinical criteria such as age (8, 1[illegible], 14, 24), duration of hypertension (8, 12, [illegible]3, 26), or radiological criteria such as kidney size, pyelogram appearance time, pyelogram density assessed on the excretory urogram (1, 15, [illegible]4, 40) and degree of stenosis and delay of the early nephrogram assessed on the renal arteriogram (51). Radioisotope renography allows accurate screening in 85% of hypertensive patients with unilateral nephropathy (28). However, its prognostic value regarding surgical curability in hypertensive patients with renovascular lesions has been assessed in very few series and without consistent results (1, 15, 24).

The prognostic value of separated renal function testing has been assessed on larger series, but even for unilateral stenosis of the main artery the results are not excellent: In a recent review Maxwell et al. (27) reported that this test allowed correct predictions in only 72% of 146 cases.

The determination of plasma renin activity (PRA) is the last proposed prognostic test. The value of its determination in the peripheral venous blood under basal conditions (supine position, normal salt diet) has been reported by Bath et al. (1) (37 cases), whereas numerous authors have emphasized the frequency with which PRA is normal in the peripheral venous blood of patients who will be benefited by operation (6, 14). On

the other hand, the determination of PRA in renal venous blood has more consistently been reported to be of value (13, 21, 31, 53).

Because of these unsettled points we undertook the present study. In this study we assessed the different prognostic criteria individually and we also tried to find the best combination of criteria on which to base prediction of the results of surgical treatment of renovascular hypertension.

MATERIAL AND METHODS

Principles of selection of patients. The first and most critical step in such a study is the selection of the patients. Because the objective of the study was to assess the value of the preoperatively available data for prediction of the blood pressure response to surgical treatment, only those patients who had undergone a technically perfect operation and who had no secondary complications were selected. The soundness of these requirements is obvious when the hypertension is cured by the operation and is probable when the hypertension is ameliorated. When there is no improvement after operation, the question arises whether the failure is related to a less-than-perfect operation or to a secondary complication, or to the fact that the renal artery lesion either was not the cause or was not the only cause of the hypertension. Therefore we excluded patients in whom there was 1) another lesion which could not be repaired; 2) a trans-stenotic gradient greater than 29 mmHg after repair; or 3) a delayed pyelocaliceal appearance of contrast medium on postoperative rapid-sequence urograms. Thus, most of the failures due to unsatisfactory operation or secondary complication have probably been excluded, with the possible exception of partial thrombosis of the graft or microemboli. However, the number of such cases may be expected to be low and, even if the bias in selection were to affect the assessment of any of the individual criteria, it should not affect comparisons of assessments. In fact, there is no reason to suppose that the selection bias would affect one criterion more than another.

A total of 43 hypertensive patients (28 men and 15 women) who had undergone operation for their renal artery lesion during the period 1964 to 1967 and in whom there was a postoperative follow-up of at least 6 months, satisfied the above criteria. Their records were reviewed. The mean age at the time of operation was 40 years (range 17 to 63 years). All patients had a stable diastolic blood pressure of 90 mmHg or greater after 3 days in the hospital. In all cases the preoperative investigation included determination of maximal urea clearance, ocular fundoscopic examination, ECG, aortorenal arteriogram, and determination of peripheral PRA. All patients had maximal urea clearances exceeding 40 ml/min.

There were unilateral main renal artery lesions in 34 cases. In two cases the lesion was unilateral and located in a primary branch of the renal artery. In seven cases the lesion was bilateral but very asymmetrical; in these cases the importance of the minor contralateral lesion in the genesis of hypertension could be excluded by the relief of the hypertension in response to repair of the more severely involved renal artery. Therefore, in this study we do not distinguish between unilateral and bilateral lesions.

Surgical treatment consisted of a revascularization procedure in 32 cases. Unilateral nephrectomy was performed in 11 cases.

The nature of the arterial lesion could be determined by histologic examination in 38 cases: atherosclerosis and thrombosis, 4 cases; neoplastic arteriovenous fistula, 1 case; aneurysm of undetermined cause, 1 case; atheromatous stenosis, 16 cases; and fibromuscular dysplasia, 16 cases.

The postoperative results were assessed according to the criteria previously proposed by Smith (43). The shortest follow-up was 6 months. However, the mean follow-up was 17 months for the cured patients, 16 months for the improved patients, and 12 months for the unimproved patients. Hypertension was considered to have been cured in patients having a systolic blood pressure of 140 mmHg or less and a diastolic blood pressure of 90 mmHg or less without antihypertensive medication or salt restriction. The patients were considered to have improved when their systolic blood pressure decreased by more than 50 mmHg and their diastolic pressure decreased to less than 110 mmHg and when the fundoscopic lesion improved without medication. The other patients were classified as unimproved. On this basis 11 patients were cured, 14 were improved, and 18 were unimproved.

Prognostic criteria studied

Clinical criteria. We assessed the prognostic value of the following clinical criteria: age of the patient at the time of operation; duration of hypertension (the period between the first sphygmomanometric measurement of a diastolic pressure greater than 90 mmHg and the operation); fundoscopic lesions as classified according to Keith et al. (22); presence of left ventricular hypertrophy as assessed by the electrocardiographic index of Sokolow; existence of a family history of diabetes mellitus or vascular disease (cerebrovascular accidents, myocardial infarction, or high blood pressure known to have occurred before the age of 60 years); and personal history suggesting a complication of atherosclerosis (cerebrovascular accident, myocardial infarction, or intermittent claudication).

Excretory urography. The excretory urogram was studied in 40 cases. It was performed in dehydrated patients in all cases; in 15 cases films were taken every minute during the first 5 min. We studied the prognostic value of the occurrence, on the side of the renal artery lesion, of the following signs: 1) decreased length (pole-to-pole diameter; a difference of 1 cm or more was considered significant in accordance with the results of Maxwell and Lupu (29)); 2) delay of 1 min or more in the pyelocaliceal appearance time of the contrast medium on rapid-sequence urograms (29); 3) increased radiodensity of medium on the 30-min urograms; and 4) pyelocaliceal "spasticity".

Radioisotope renography. This study was performed with the Alvar renograph (49). The only parameter assessed was the peak time because it has been proved to have the smallest variance (56). The value of the following two criteria was studied: 1) delay of the peak on the side of the lesion, expressed in percentage of the contralateral peak time

$$\frac{\text{ipsilateral peak time} - \text{contralateral peak time}}{\text{contralateral peak time}} \cdot 100$$

and 2) the absolute value of the contralateral peak time.

Renal arteriography. The method of Seldinger (42) was used. The prognostic value of the degree of stenosis and of the presence of hemodynamic delay was studied. The degree of stenosis was evaluated by measurement of the diameter of the stenosis and of the arterial lumen above the stenosis. Three degrees are distinguished: stenosis = 50%, > 50%, or < 50%. Hemodynamic delay was considered to be present when one of the following signs was observed (51): 1) delay in the filling of the renal artery and its branches compared with the contralateral renal artery and the aorta; 2) delayed appearance of the early nephrogram; and 3) stasis beyond the stenosis. The correct position of the catheter 2 cm above the renal arteries, was always checked before these signs were interpreted as evidence for hemodynamic delay.

Separated renal function. This study was performed under conditions of water diuresis. The patients ate a normal diet and discontinued diuretic and hypotensive drugs 8 days before the test. Twenty-one patients were investigated but the test was technically satisfactory in only 18. For each parameter the comparison between the two sides was made as follows:

$$(V_{DK} - V_{N})/V_{N} \cdot 100$$

in which V_{DK} is the value for the diseased kidney and V_{N} is the value for the normal or less diseased kidney. The prognostic value of the percentage difference in urine flow, urinary sodium concentration, and urinary creatinine (or inulin) concentration was studied.

Plasma renin activity. PRA was measured by the method of Boucher et al. (4) under basal conditions: the patient was on a normal salt diet (120 mEq of sodium), without any diuretic or hypotensive drug, for 1 week and in a recumbent position for 10 hours before sampling. The results were expressed in nanograms of angiotensin produced by 1 l of plasma during 1 min of incubation. The reproducibility of the method has been previously reported (30): the variation of repeated measurements on one plasma sample is less than 15%. The mean value of peripheral PRA in normotensive subjects, under basal conditions, is 13.8 ng/l/min (S.D. = 6.9; range 6 to 33). PRA was measured in the peripheral blood of the 43 patients in this study. In 25 patients PRA was also measured in renal venous blood and, in 20 of these patients, PRA was measured in the inferior vena cava (IVC) above the renal veins. The blood samples were obtained by retrograde catheterization of the IVC according to a procedure which has been previously reported (30). The prognostic value of the following was assessed: 1) PRA of peripheral venous blood and of blood from the IVC above the renal veins; and 2) ratio of PRA in the renal venous blood from the diseased side to PRA in the renal venous blood from the normal or less diseased side.

Histology. The histologic features of the diseased kidney were studied in 36 cases, most frequently on tissue obtained by needle biopsy during the operation. The slides were prepared according to standard techniques which have been previously reported (39).

The juxtaglomerular apparatus was examined in only 14 cases, because in 22 specimens there was insufficient cortex, a not infrequent occurrence with needle biopsy (35). In man the hypergranularity of the juxtaglomerular cells is a poor index of their activity (35) so we considered only hyperplasia of the juxtaglomerular apparatus. We did not count the cells but only evaluated the presence or absence of hyperplasia (2).

The prognostic value of assessment of the degree of arteriolar sclerosis and interstitial fibrosis was also studied. The degree of arteriolar sclerosis was evaluated according to the criteria of Sommers et al. (44): stage 0, no lesion; stage 1, focal thickening of the arterioles; stage 2, arteriolar wall thickness equal to the luminal diameter; and stage 3, arteriolar wall thickness greater than the luminal diameter. The degree of interstitial fibrosis was assessed, in tissue stained with Masson trichrome, according to the classification of Bariety et al. (2) in three stages: no or minimal fibrosis, mild fibrosis, and severe fibrosis.

The actual data obtained in the various tests used as criteria have been previously reported (14).

Statistical methods. Our patients were classified into two groups according to the surgical results: improved (includes cured) and unimproved. The statistical analysis was performed in three steps: 1) the prognostic value of each criterion was assessed individually; 2) the prognostic value of each investigation was studied, taking into account all the criteria which could be determined in one type of investigation; and 3) by means of discriminant analysis the group of criteria was sought which would best predict improvement or lack of improvement.

Individual study of criteria. The qualitative criteria are those which could be defined only by their presence or absence. Each criterion was chosen so that a positive relationship was expected between its presence and surgical improvement. When the presence of the criterion was associated with improvement or its absence was associated with lack of improvement, the instance was called "correctly classified" ("true criterion"). The instance was called "incorrectly classified" when the presence of the criterion was associated with failure (false positive) or when its absence was associated with improvement (false negative). The prognostic value of each criterion was then assessed by the chi-square test. Only when this test showed significance was the percentage of correctly classified patients calculated.

The quantitative criteria were assessed by two kinds of tests.

1. They were first assessed directly by the test of Mann and Whitney (or Wilcoxon test) (50). Student's test was not used because the variances between the groups of improved patients and unimproved patients were not identical.

Then they were assessed by the chi-square test. For that purpose the quantitative criteria were transformed into qualitative criteria by dichotomizing the variable about a value chosen as being an appropriate threshold. The threshold value for age, duration of hypertension, PRA, and ratio of PRA between the renal veins was chosen in order to get the highest chi-square value. For the disparity of the peak time, 50% was taken as threshold value because it has been proved to be significant by Weeden et al. (52). For the contralateral peak time, 5 min was chosen as threshold value because Tapia (48) has reported it to be the maximal value for normal kidney under conditions of dehydration. Decreases of 50% for the urine flow and of 15% for the urinary sodium concentration were taken according to the criteria proposed by Howard and Connor (18). For the urinary concentration of creatinine or inulin we chose 30% as the threshold according to the criteria of Gordon et al. (16).

Prognostic value of complex investigation. Some investigations, like the separated renal function study and excretory urogram, allow determination of many criteria. The best way to assess the prognostic value of any complex investigation on the basis of all the available criteria was sought in two directions: 1) by assessing the prognostic value of the requirement of the presence of any one criterion, and 2) by assessing the prognostic value of the requirement of the simultaneous presence of two or more criteria. For the separated renal function study the two combinations of criteria assessed are those of Howard and Connor (18) and Rapoport (38).

Discriminant analysis. This analysis allows classification of a given patient by using concomitantly all the measurements made on that patient (37). For that purpose the measurements already made on a group of surgically treated patients are used to calculate an expression called the discriminant function. This function is a linear combination of the measurements. It is so calculated that it is expected to be positive for the improved patients and negative for the unimproved patients. To be used, qualitative criteria had first to be quantified by giving them the value 1 when present and 0 when absent.

For any new patient the results of the various determinations are introduced into the discriminant function. The new patient may be considered as likely to be improved when the function is positive and as likely to be unimproved when the function is negative.

To classify the criteria according to their prognostic value, we proceeded stepwise. We first sought the isolated criterion which was the most powerful discriminator and then the most powerful pair of discriminators.

The percentage of patients correctly classified has been given for each step. Because of the small size of our sample, these percentages are only an approximate index of the accuracy of prediction for new patients. The number of criteria which may be justifiably inserted into the discriminant function depends on the size of the sample, so we have taken into account only those criteria considered to be important. Since all the criteria could not be determined in all patients, many analyses were required.

RESULTS

Prognostic Value of Qualitative Criteria

The qualitative clinical criteria (funduscopic classification, family history of vascular disease or diabetes, history of cerebrovascular accident, myocardial infarction or leg claudication, and the presence of abdominal bruit) were found to have no prognostic value (Table I).

Excretory urographic criteria. The delay of the pyelocaliceal appearance of contrast medium was evaluated only in the 15 cases in which rapid-sequence early films were available. The presence of such a delay was a good prognostic criterion ($p < 0.01$). This criterion correctly predicted the results of operation in 80% of the cases.

Pyelocaliceal contrast medium density and spasticity were assessed in 37 cases (in 3 other cases there was no excretion of contrast medium on the diseased side). Hyperdensity of the contrast medium films was of prognostic value ($p < 0.05$): it predicted the results of operation in 65% of the cases. However there were numerous false negative (11 cases) and a few false positive (7 cases) results. There was no prognostic value for spasticity.

Size disparity of 1 cm or greater had prognostic value ($p < 0.05$): it predicted the results of operation in 68% of the cases.

Renal arteriography. Hemodynamic delay was always present when the degree of renal artery stenosis was greater than 50% and always absent when renal artery stenosis was less than 50%. This sign had a good prognostic value ($p < 0.01$): it correctly predicted the results of operation in 71% of the 42 cases in which it was evaluated. When hemodynamic delay was studied in the 19 cases having approximately 50% stenosis, the prognostic value of this sign was not of statistical significance.

Histology. The pathologic classification of the renal artery lesion was accomplished in 32 cases. In the group of 16 patients having fibromuscular dysplasia, 13 were cured or improved. Of the 16 patients having atherosclerotic renal artery stenosis, 8 were cured or improved. The apparent difference in prognosis between the two types of lesions is not statistically significant in this series.

The presence of hyperplasia of the juxtaglomerular apparatus, assessed in 14 ipsilateral biopsies, had no prognostic value.

Table I. *Prognostic value of qualitative criteria*

Criteria	Cases studied (no.)	Criterion present: Improved ("true")	Criterion present: Unimproved ("false positive")	Criterion absent: Improved ("false negative")	Criterion absent: Unimproved ("true")	p^a	Total percentage correctly classified[b]
Clinical criteria							
Fundus normal or grade I or II	43	15	8	10	10	NS	—
Absence of L. ventricular hypertrophy	42	11	8	13	10	NS	—
Absence of family history of CVA or diabetes mellitus	37	9	7	14	7	NS	—
Absence of CVA	43	20	12	5	6	NS	—
Abdominal bruit	34	10	4	11	9	NS	—
Excretory urography							
Size disparity > 1 cm	40	16	6	7	11		68
Delayed pyelocalyceal appearance time	15	6	3	0	6		80
Late pyelogram hyperdensity	37	10	2	11	14		65
Pyelocalyceal spasticity	37	9	3	12	13	NS	—
Renal arteriography							
Hemodynamic delay							
All cases	42	20	7	5	10		71
50% stenosis	19	7	3	4	5	NS	—
Histologic features							
Fibromuscular dysplasia	32	13	3	8	8	NS	—
Ipsilateral juxta-glomerular hyperplasia	14	5	4	3	2	NS	—
Ipsilateral arteriolosclerosis, stage 0 or I							
All cases	36	14	8	8	6	NS	—
Repaired functional stenoses	21	11	1	7	2	NS	—
Ipsilateral interstitial fibrosis, non-severe							
All cases	36	19	8	3	6	NS	—
Repaired functional stenoses	21	18	0	0	3		100

[a] By chi-square test.
[b] Percentages given only for criteria showing significance on chi-square test.
NS = not significant.
<0.05 <0.01.

The degree of arteriolonephrosclerosis and interstitial fibrosis of the ipsilateral kidney tissue was assessed in two groups. The first group comprised all patients from whom tissue was obtained. The second group comprised only patients having reconstructive surgery for renal artery lesions which were presumed to be functionally significant on the basis of the following criteria: delay of the pyelocalyceal appearance time of contrast medium on the excretory urogram, hemodynamic delay on the renal arteriogram, delay of the peak time on the radioisotope renogram, increase in the urinary creatinine concentration by more than 30% on the affected side in separated renal function tests, and PRA in the suprarenal IVC greater than 33 ng/l/min. In neither group did the degree of arteriolonephrosclerosis have prognostic value. The degree of interstitial fibrosis had no prognostic value in the entire group of patients but did have in the group of patients whose functionally significant renal artery was repaired.

Table II. *Prognostic value of quantitative criteria*

Criteria	Cases studied: Improved	Cases studied: Unimproved	Mann-Whitney test	Threshold, expected improvement	Criterion present: Improved ("true")	Criterion present: Unimproved ("false positive")	Criterion absent: Improved ("false negative")	Criterion absent: Unimproved ("true")	Chi-square test	Total percentage correctly classified[a]
Duration of hypertension	25	18		< 1.5 y	13	3	12	15		65
Age	25	18	NS	40 y	15	5	10	13		65
Renogram										
Delay of peak time	14	13	NS	> 50	9	6	5	7	NS	—
Contralateral peak time	14	13	NS	< 5 min	13	10	1	3	NS	—
Separated renal function										
Decreased urine flow	10	7	NS	> 50 %	6	2	4	5	NS	—
Decreased Na	10	4	NS	> 15 %	5	2	5	2	NS	—
Increased creatinine or inulin	11	7		> 30 %	10	2	1	5		83
PRA										
Peripheral	25	18	NS	> 33 ng/l/min	9	6	16	12	NS	—
Renal venous ratio	11	14	NS	1.5	7	5	4	9		64
Suprarenal IVC	7	13		> 33 ng/l/min	6	2	1	11		85

[a] Percentages given only for criteria showing significance on chi-square test.
NS = not significant.
* $p < 0.05$ ** $p < 0.01$

Prognostic Value of Quantitative Criteria

Age. Although the mean age (38 years) of the improved patients was less than the mean age (42 years) of the unimproved patients, the difference is not significant according to the Wilcoxon test (Table II). However the chi-square test shows that improvement is significantly more frequent in patients less than 40 years old.

Duration of hypertension. This criterion had prognostic value with both statistical tests.

Radioisotope renogram. Neither of the criteria obtained by examination of the radioisotope renogram had significant prognostic value with either of the two statistical tests.

Separated renal function tests. In 17 cases, the urine flow was measured accurately. A reduction of 50% on the decreased side did not prove to be a good prognostic criterion. In the 14 cases in which urinary sodium concentration was measured accurately, a decrease in urinary concentration of 15% or more had no prognostic value. On the other hand, in the 18 cases in which urinary creatinine or inulin concentrations were measured, an increase of 30% or more was found to be a good prognostic criterion ($p < 0.05$). This criterion allowed correct prognostic classification in 83% of the cases.

Plasma renin activity. Measurement of PRA in the peripheral blood was of no prognostic value with either statistical test. The ratio of PRA between the renal veins had no significant prognostic value with the Mann and Whitney test, but, *by the chi-square test, a ratio of 1.5 or greater* was found to be significantly related to improvement. Significant prognostic value was found with both statistical tests for PRA in the suprarenal IVC: the threshold of 33 ng/l/min allowed correct prediction of surgical results in 85% of the cases.

Prognostic Value of Complex Investigation

Excretory urography. Association of a size disparity of 1 cm, in the standard urogram, with delay of the pyelocaliceal appearance time, hyperdensity of the late pyelogram, or spasticity on the pyelogram, led to such an increase in the num-

Table III. *Prognostic value of combined criteria evaluated in one investigation*

Criteria	Cases studied	Criteria present: Improved ("true")	Criteria present: Unimproved ("false positive")	Criteria absent: Improved ("false negative")	Criteria absent: Unimproved ("true")	p^a	Total percentage correctly classified[b]
Excretory urogram							
Standard urogram							
One criterion only	25	13		4	6		76
Size decrease + delay PCAT or hyperdensity or spasticity	25	8	1	9	7	NS	—
Rapid-sequence urogram							
Delay PCAT + hyperdensity	15	5	1	1	8		86
Delay PCAT + hyperdensity + size decrease	15	4	1		8		80
Isotope renogram							
Delayed ipsilateral peak time > 50% + contralateral peak time ≤ 5 min	27	9	4	5	9	NS	—
Separated renal function study							
Howard & Connor (18)	14	5	0	5	4	NS	—
Rapoport (38)	14	7		3		NS	—
PRA							
Renal vein ratio ≥ 1.5 + suprarenal IVC PRA ≥ 33 ng/l min	20	4	2	3	11		75

[a] By chi-square test.
[b] Percentages given only for criteria showing significance on chi-square test.
PCAT = pyelocaliceal appearance time
NS not significant.
< 0.05 < 0.01.

ber of false negative results that these combinations of two criteria had no prognostic value (Table III). On the other hand, when only one sign was required, the number of false negatives was only four and this criterion had a good prognostic value, correctly predicting the results of operation in 76% of the cases.

In the rapid-sequence urogram the association of a delay in the pyelocaliceal appearance time and hyperdensity of the late pyelogram had a good prognostic value ($p < 0.01$ correct classification of 86% of the cases). The additional requirement of a size discrepancy of 1 cm did not improve the prognostic value of the above combined criteria, and it led to two false negative results rather than one.

Radioisotope renogram. The combination of the two criteria evaluated by radioisotope renography had no significant prognostic value.

Separated renal function studies. The criteria of Howard and Connor (18) and Rapoport (38) appear to have no significant prognostic value.

Plasma renin activity. When the criterion of a ratio of PRA in the renal venous samples of 1.5 or greater was added to the criterion of a PRA in suprarenal IVC blood of 33 μg/l min or greater the percentage of correctly classified patients decreased from 85% (Table II) to 75% (Table III).

Discriminant Analysis of Multiple Criteria

The first two discriminant analyses evaluated the criteria which are usually available to the clinician before renal arteriography is undertaken.

Table IV *Discriminant analysis*

No. of pats.	Criteria: Rejected	Criteria: Discriminant	Units	Mean: Unimproved	Mean: Improved	Percentage correctly classified
1st discrimination						
17 unimproved 23 improved	Age; fundus	Duration of hypertension (*d*)	Mo.	101	34	(*d*): 68
		Diastolic pressure (*p*)	cmHg	10.9	11.8	(*d*)+(*p*): 78
		EU (*u*) – sum of criteria	0 to 4	0.94	1.83	(*d*)+(*p*)+(*u*): 80
	1st discriminant function for the improved: $0.59p + 0.27 - 0.014d - 6.1 > 0$					
2nd discrimination						
12 unimproved 12 improved	Age; fundus; diastolic pressure Δ peak time (ΔPT) > 50 %; contralateral peak time (CPT) < 5 min	Duration of hypertension	Mo.	109	30	(*d*): 66
		EU	0 to 4	1.1		(*d*)+( ): 71
	2nd discriminant function for the improved: $0.38 - 0.012d + 0.26 > 0$					
3rd discrimination						
17 unimproved 23 improved	Age; fundus, EU diastolic pressure degree of stenosis	Delay on arteriogram (*a*)	1 if present, 0 if not	0.4	0.9	(*a*): 78
		Duration of hypertension	Mo.	101	34	(*a*) (*d*): 85
	3rd discriminant function for the improved: $2.9a - 0.012d - 1.12 > 0$					
4th discrimination						
12 unimproved 12 improved	Age; fundus; diastolic pressure; EU ΔPT > 50 %; CPT > 5 min	Delay on arteriogram	1 or 0	0.3	0.9	(*a*): 79
		Duration of hypertension	Mo.	109	30	(*a*) (*d*): 87
	4th discriminant function for the improved: $3.7 - 0.017d - 1.3 > 0$					
5th discrimination						
13 unimproved 7 improved	Delay on arteriogram duration of hypertension	PRA in supra-renal IVC (*r*)	ng/l/min	26.2	70.3	80
	5th discriminant value for the improved: > 48.2					
6th discrimination						
7 unimproved 11 improved	Delay on arteriogram duration of hypertension	Increased urinary creatinine on diseased side (Δ cr)	1 = 15 % 2 = 16–29 % 3 = 30–99 % 4 = 100 %	1.86	3.45	83
	6th discriminant percentage for the improved: Δcr > 30 %					

EU = excretory urographic criteria (see text).

In the first analysis (Table IV) five criteria observed in 40 patients were evaluated: age, duration of hypertension, diastolic blood pressure, funduscopic changes, and results of excretory urography. The urographic criteria were summarized by assigning a number 0 to 4 according to the number of simple criteria present in each case (sum of criteria). The best single discriminant criterion was the duration of hypertension: it allowed correct classification of 68 % of the patients. The best pair of criteria were the duration of hypertension and the diastolic blood pressure, allowing correct classification of 78 % of the patients. The sum of criteria of the excretory urogram was the third best prognostic criterion and, when combined with the preceding two, allowed correct classification in 80 % of the cases.

In the second discriminant analysis the above

criteria were evaluated with the two criteria of the radioisotope renogram. These seven criteria were evaluated in 24 patients. In this analysis also, the duration of hypertension remained the best single discriminant criterion (allowing correct classification in 66% of the cases). The best paired criteria were the duration of hypertension and the sum of criteria of excretory urography (allowing correct classification in 71% of the cases).

In the third and fourth analyses we added the two variables evaluated by arteriography (hemodynamic delay and the degree of arterial occlusion) to the variables studied in the first two analyses. The third and fourth analyses gave the same result: the best discriminant variable was the hemodynamic delay (78% of patients classified correctly), and the best pair was the combination of hemodynamic delay and duration of hypertension (85% of patients correctly classified).

In the fifth discriminant analysis we compared the PRA in the suprarenal IVC and the two best discriminant criteria thus far identified, the hemodynamic delay and the duration of hypertension. The suprarenal PRA proved to be a better discriminant than the other two. Alone it allowed correct classification of 80% of the patients. Because the sample size was small (20 patients), no best discriminant pair was determined. The threshold value of the suprarenal IVC PRA (allowing discrimination of the improved group of patients with 80% accuracy) was 48.2 ng/l/min.

In the sixth discrimination we compared the increase of the urinary concentration of creatinine or inulin on the diseased side with the hemodynamic delay and the duration of hypertension. For statistical purposes the increase in urinary creatinine was classified in four stages (Table IV). Increased urinary creatinine concentration proved to be the best discriminant criterion, allowing correct classification in 83% of the cases.

Example of the interpretation of discriminant functions. The first discriminant function (Table IV) is $0.59 \times$ diastolic pressure (cmHg) $+ 0.27 \times$ criteria on excretory urography $- 0.014 \times$ duration of hypertension (mo.) $- 6.1$ and is evaluated as follows. If a patient has a diastolic blood pressure of 110 mmHg for 10 months and his excretory urogram yields two criteria, the value of the discriminant function will be $0.59 \times 11 + 0.27 \times 2 - 0.014 \times 10 - 6.1 = 0.79$. The discriminant function for this patient is positive, so we would conclude that he is likely to be benefited by surgical treatment.

DISCUSSION

Clinical criteria. Hypertension which is severe and of short duration is most likely to be relieved by operation. This is in agreement with the reports by Maxwell et al. (26) and Bath et al. (1) that renal artery stenosis is frequently discovered in patients having a recent onset of severe hypertension. A history of vascular accident does not indicate that the patient will have a poor response to operation. That does not mean, however, that severity of atherosclerosis has no prognostic value with regard to the survival rate. On the contrary, it has been recently shown (55) that the survival rate of patients with atheromatous renovascular hypertension was not improved by operation, despite the decrease in blood pressure, because this group of patients experienced a high rate of cerebrovascular accident or myocardial infarction postoperatively. Although the presence of an abdominal bruit did not prove to be of prognostic value, this does not negate the diagnostic value of bruits of renovascular origin. The finding of a highpitched continuous or systolic-diastolic bruit beneath the costal margin or lateral to the lumbar spinal region is a good diagnostic clue of significant renal artery stenosis (19).

Excretory urography and renal arteriography. When only a standard excretory urogram is available, it is advisable to require the presence of only one criterion, because the combination of two or more criteria leads to numerous false negative results. On the other hand, when a rapid-sequence urogram is available, the combination of delay in the pyelocaliceal appearance time of contrast medium and hyperdensity in the late pyelogram has the best prognostic value. In this series hyperdensity of the late pyelogram had a high incidence of false negative results, undoubtedly due to the fact that the urograms were not obtained under the optimal conditions to unmask the phenomenon (water load (6), mannitol infusion (54), or urea infusion (47)).

The renal arteriogram produces not only ana-

tomic information which is absolutely necessary for the surgeon, but also functional information having prognostic value Hemodynamic delay was evaluated primarily on the basis of a delay in the appearance of the early nephrogram during renal arteriography This delay can also be evaluated by excretory urography when a double dose of contrast medium is injected rapidly (less than 8 sec) (29) and when films are taken as early as 30 sec after injection. If minute-sequence excretory urography is then carried out during the first 5 min after beginning injection and then urea is infused by the technique of Stejskal et al. (47), all the excretory urographic criteria of ischemia can be evaluated. It is possible that an adequate combination of all these criteria will prove to have better prognostic value than the arteriogram. That these combined criteria will be useful is suggested by the results in this limited series of 15 cases, of which 13 were correctly classified by using the association of delay of pyelocaliceal appearance time and hyperdensity of the late pyelogram.

Radioisotope renogram. The two criteria evaluated on the radioisotope renogram proved to have no prognostic value when considered individually in association, or by discriminant analysis, in agreement with the report by Bath et al. (1) but disagreeing with reports by Gammelgaard et al. (15) and Levitt et al. (24). However the criteria chosen in this study were not exactly comparable with those of the last two reported studies.

Separated renal function study Among the various parameters used in this study to assess the renal functional asymmetry only urinary concentration of creatinine (or inulin) had prognostic value, in agreement with findings by Stamey (45) and Guedon et al. (16), who stressed the importance of the increased concentration of nonreabsorbable solute on the ischemic side, as well as with the findings of Hunt et al. (19) who emphasized the increased reabsorption of water Discriminant analysis also showed that the increase in urinary creatinine concentration is of great prognostic value and is a better prognostic criterion than either hemodynamic delay or duration of hypertension. The urinary concentration of sodium had no prognostic value because of the large number of false negative results. This explains why the criteria of Howard and Connor (18) and Rapoport (38) which take into consideration the urinary sodium concentration, had no diagnostic value in this series.

Plasma renin activity The inferiority of the ratio of PRA in the renal veins, compared to PRA in the suprarenal IVC, was shown, in our series of 20 patients who had both determinations, by the occurrence of two more false positive and two more false negative results. The two false positive results might be explained by an increase of PRA concentration due to a decrease in renal blood flow alone without actual concomitant hypersecretion of renin (30) The two false negative results may be explained by the contamination of renal blood with gonadal venous or IVC blood they cannot be explained by immediately previous stimulation of renin secretion (3_) because the patients were recumbent for 10 hours before the test and because they showed no decrease in blood pressure before sampling. It has been recently shown (20) that renin hypersecretion by an ischemic kidney may be masked by a normal diet and unmasked by sodium depletion. This fact may account for false negative results in determination of the ratio of PRA between the renal veins, but not for our false negative results, because suprarenal PRA was concomitantly high in our cases.

The numerous false negative results with PRA in peripheral blood are in disagreement with Bath et al. (1), who reported that increased PRA in basal conditions allowed correct classification in 96% of patients with unilateral renal artery stenosis. However many other authors have stressed the high incidence of false negative results based on peripheral PRA in basal conditions. Cohen et al. (7) suggested orthostatic maneuvers to unmask more accurately the hypersecretion of renin.

Renal histology The finding that hyperplasia of the juxtaglomerular apparatus had no prognostic value agrees with that of Barajas et al. (2).

The more severe the interstitial fibrosis, the greater the chances that surgical repair of the artery will not cure the hypertension. It may be useful to perform renal biopsies preoperatively in patients for whom revascularization procedures would otherwise be recommended because of functionally significant renal artery stenosis. Further experience may confirm these preliminary observations, suggesting that nephrectomy would be necessary in the face of severe interstitial fibrosis. However present data are based on insufficient

numbers of cases to permit this recommendation now

Relative importance and usefulness of preoperative investigations. The combination of clinical and roentgenologic data allowed correct prediction in 80% of the cases when the criteria were duration of hypertension, diastolic blood pressure, and urographic features, and in 85% when the criteria were duration of hypertension and presence of hemodynamic delay. These are good percentages compared to those found with the two best individual criteria: 80% with PRA in the suprarenal IVC and 83% with increase in urinary creatinine or inulin concentration. This emphasizes the value of the preoperative clinical and roentgenologic observations. However this does not mean that the more sophisticated studies, such as separated renal function and catheterization of the IVC for PRA determinations, are not useful. When included in a discriminant analysis with the clinical and roentgenologic data, these latter two criteria appear to provide the best discrimination. In the present study the number of patients who had undergone such procedures was small, so it was statistically meaningless to calculate a discriminant function with all the criteria. Therefore, we could not answer the following two questions:

1. What accuracy of prediction could be achieved by combination of the five best criteria

2. Is it justified to perform bilateral ureteral catheterization and IVC catheterization in order to increase the accuracy of prediction.

For the present, the use of these two procedures seems to be perfectly justified, and all the more because their prognostic value may be increased—for example, the prognostic value of the renal vein PRA is increased by stimulation by salt depletion (20), and assessment of blood flow in the contralateral kidney has been found to improve the predictive bilateral ureteral catheterization (46).

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HEMODYNAMIC EFFECTS OF ADRENALINE, NORADRENALINE AND ISOPROPYLNORADRENALINE IN PATIENTS WITH A FIXED RATE ARTIFICIAL PACEMAKER

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Abstract. The hemodynamic response in four patients with an artificial pacemaker stimulating at a fixed rate has been investigated during intravenous infusions of equimolar dosages of adrenaline, noradrenaline and isopropylnoradrenaline. The infusion rate was increased at 8 min intervals to give dose-response curves. Adrenaline produced an increase of atrial rate, cardiac output, stroke volume, mean pulmonary artery and PCV pressure while the mean aortic pressure was unchanged or slightly decreased. The noradrenaline produced no change in atrial rate, cardiac output or stroke volume, while the mean pulmonary artery pressure as well as the mean PCV and aortic pressure increased. The isopropylnoradrenaline infusion produced rise in atrial rate, cardiac output and stroke volume, while no change or slight fall was observed in mean pulmonary artery, PCV and aortic pressures.

Patients with a fixed rate artificial pacemaker may run into the situation of needing vasopressor agents such as noradrenaline. It is therefore important to know how these patients with a constant pulse rate respond to drugs, many drugs act via changes in pulse rate. Such patients also offer a unique possibility of studying the effect of drugs when the chronotropic effect is excluded.

Four patients with a fixed rate pacemaker have been investigated and the dose-response curves of adrenaline, noradrenaline and isopropylnoradrenaline in equimolar dosages have been studied from a hemodynamic point of view.

CASE REPORTS

Case 1

Male, born in 1895. Well until 1960, when dyspnea on exertion started, as did nocturnal cough. In 1963 slow pulse and systolic hypertension, 40 mmHg, were observed. He fainted several times in 1964. Admitted to hospital and found to have complete heart block, right and left bundle branch block intermittently and an enlarged heart with total volume in systole of 930 ml and in diastole of 1 060 ml. The corresponding volumes per m² BSA were 480 and 550 ml. Through thoracotomy Medtronic fixed rate pacemaker was implanted in 1964, and his syncopal attacks disappeared. The pulse rate averaged 72/min.

Case 2

Male, born in 1888. Well until 1963, when he had first attack of dizziness; he also noted a slow pulse, and an ECG showed 2:1 and 3:1 A-V block. His heart was enlarged with total volume in systole of 1 200 ml and in diastole of 1 360 ml, resulting in systo-diastolic difference of 15%, which is more than normal. His pulse rate fell sometimes to 30/min and was sometimes combined with Adams-Stokes attacks. He was given Medtronic fixed rate pacemaker with stimulation rate of 60/min through thoracotomy and his Adams-Stokes attacks disappeared. Because of malfunction, pacemaker was later inserted via the jugular vein to the right ventricle.

Case 3

Male, born in 1893. Well until 1962, when he noticed slow pulse, and the ECG showed second degree A-V block changing to complete heart block with pulse rate of 35/min. The bradycardia resulted in angina pectoris and moderate symptoms of cardiac decompensation with dyspnea and tiredness. An X-ray of the heart showed volume of 1 060/600 ml/m² BSA. The A-V block appeared intermittently as did Adams-Stokes attacks. He was given fixed rate Medtronic pacemaker with epicardial electrodes through thoracotomy in 1964 and his condition improved.

Case 4

Male, born in 1904. Apart from pneumonia in 1945 he was well until 1959 when he became tired and dyspneic on exertion. He then had repeated bouts of complete heart block with pulse rate around 30/min and

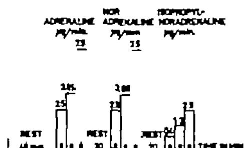


Fig 1 Time schedule and the various catecholamine concentrations. The infusion was started 40 min after the catheters had been inserted. Adrenaline was then infused at a concentration of 2.5 μg/min for 8 min, followed by 3.85 μg min for another 8 min, and 7.5 μg min for 8 min again. The adrenaline infusion was then stopped, and followed after 20 min by the nor adrenaline infusion.

Adams-Stokes attacks. He was hospitalized in 1964 his ECG showed complete heart block and left bundle branch block. His heart volume was 940 ml in systole and 1 170 ml in diastole, corresponding to 540 and 680 ml/m² BSA at a pulse rate f 34/min. A Medtronic fixed rate pacemaker was implanted through a thoracotomy in March 1964 and he has felt well since then, with no Adams-Stokes attacks.

METHODS

The clinical procedure has been reported in detail elsewhere (6). The catheterization was performed several months after the pacemaker implantation. I cases 1 and 3 comparative catheterization was performed before the pacemaker implantation while the patient had complete heart block. The patients arrived for the hemodynamic study in the morning after a light breakfast. No premedication was given. The patients were in the supine position. Arterial catheters were inserted by the Seldinger technique. A catheter PE 160, was introduced into the brachial artery and teflon catheter into the ascending aorta, with the aid of fluoroscopy. Another PE 160 catheter was introduced percutaneously via an arm vein to the right atrium. Through venous incision double lumen Cournand catheter was manipulated into the pulmonary artery and the tip was left in the wedge position; in case 3 brachial artery instead of aortic recordings were obtained, and in case 2, who had pacemaker wire inserted via the jugular vein, no catheter was introduced into the pulmonary artery.

ECGs and pressure curves were recorded with a four channel direct-writing ink jet electrocardiograph (Mingograph 81 Elema-Schönander Stockholm). Mean pressures were obtained by electrical integration over period including at least two respiratory cycles. Zero level for the strain gauges was the mid-thoracic line measured at the sternal insertion of the fourth rib. Cardiac output (CO) was determined by dye dilution technique (11). Hematocrit was determined in duplicate on arterial blood drawn from an Ellermann tube into 75 mm heparinized microhematocrit tube and centrifuged at 8 000 rpm for at least 5 min.

The left ventricular minute work in kgm/min was calculated according to the following formula:

$$\frac{CO \times (\bar{P}_{AO} - \bar{P}_{PCV}) \times 13.6}{1\,000}$$

where CO is the cardiac output, $\bar{P}_{AO}$ the mean aortic pressure and $\bar{P}_{PCV}$ the mean PCV pressure. The left ventricular stroke work in grammeters was calculated according to the formula

$$\frac{SV \times (\bar{P}_{AO} - \bar{P}_{PCV}) \quad 13.6}{1\,000}$$

where SV is the stroke volume. The right ventricular minute work and right ventricular stroke work were calculated similarly using the mean pulmonary and the mean right atrial pressures ($\bar{P}_{RA}$) instead of $\bar{P}_{AO}$ and $\bar{P}_{PCV}$, respectively.

The pulmonary vascular resistance (PVR) expressed in dyn sec cm⁻⁵ was calculated as the difference between $\bar{P}$ and $\bar{P}_{PCV}$ in mmHg divided by CO in ml/sec and multiplied by 1 332 (1). The systemic resistance was calculated in the same way substituting $\bar{P}$ by $\bar{P}_{AO}$ and $\bar{P}_{PCV}$ by $\bar{P}_{RA}$.

Pressures were recorded at frequent intervals until a steady state was obtained. Resting CO was obtained 30 and 40 min after the catheter had been put in place. An adrenaline infusion was now started with the aid of an infusion machine; the lowest concentration was 2.5 μg/min, the next was 3.85 μg/min and the highest concentration was 7.5 μg/min (Fig. 1). Each concentration corresponding to equimolar dosages of the three catecholamines (as base) was infused for 8 min, after which the infusion rate could easily be changed to the next higher one. During each 8 min period pressures were recorded at 2-min intervals, and CO was determined at the end of each 8-min period.

After 8 min on the highest adrenaline concentration

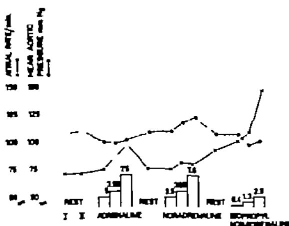


Fig. 2 Mean atrial rate (beats/min) and mean aortic pressure (mmHg) during rest and catecholamine infusion.

the adrenaline infusion was stopped and substituted by slow saline infusion. After 15 to 20 min, when pressures and flow had returned to the original values, the next infusion was started—noradrenaline (case 1) or isopropylnoradrenaline (case 2). In cases 3 and 4 noradrenaline was given first, followed by an isopropylnoradrenaline infusion. The noradrenaline and isopropylnoradrenaline concentrations are given in Fig. 1.

RESULTS

Patients with a Fixed Rate Pacemaker

Rather pronounced differences between the patients were noted for some parameters, but the responses to the various drugs were similar in all patients.

The *mean atrial rate* (Fig. 2) was 71/min both 30 and 40 min after the catheters had been put in place, indicating that the patients were in a steady state when the catecholamine infusion started. During the adrenaline infusion the atrial rate rose slightly to 75/min at a concentration of 2.5 μg/min, rising further to 86 and 97/min at 3.85 and 7.5 μg/min, respectively. This is in contrast to the largely unchanged atrial rate during the noradrenaline infusion and the pronounced rise during the isopropylnoradrenaline infusion—up to 148/min at an infusion rate of 2.5 μg/min.

The *mean aortic blood pressure* (Fig. 2) rose during the noradrenaline infusion from 108 to 123 mmHg at the highest concentration but showed a tendency to decrease during the adrenaline—from 110 to 102 mmHg—and the isopropylnoradrenaline infusions—from 108 to 101 mmHg. Looking at the pulse pressure, however

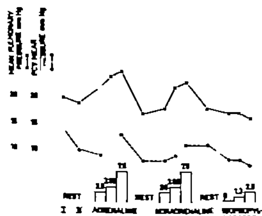


Fig. 3. Mean blood pressure for mean pulmonary artery and PCV (mmHg) during rest and catecholamine infusions.

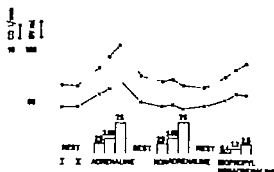


Fig. 4. Cardiac output (l/min) and stroke volume (ml) during rest and catecholamine infusions.

all three drugs produced an increase, adrenaline from 47 at rest to 61 mmHg at the highest concentration the corresponding values for noradrenaline were 40 and 71 mmHg, and for isopropylnoradrenaline 45 and 62 mmHg. This change was produced during the adrenaline and isopropylnoradrenaline infusions, mainly by a decrease of the diastolic pressure, whereas the noradrenaline raised both the diastolic and the systolic pressure, the systolic rise being more pronounced.

The *mean pulmonary artery blood pressure* (Fig. 3) showed an increase of about the same magnitude during both the adrenaline and the noradrenaline infusion, while a tendency to fall was noted during the isopropylnoradrenaline infusion. The pulse pressure in the pulmonary artery increased, however the rise during the adrenaline infusion being from 17 mmHg at rest to 28 mmHg at the highest concentration, during noradrenaline from 17 to 24 mmHg, and during isopropylnoradrenaline from 16 to 24 mmHg. This rise in the pulse pressure was produced during the adrenaline infusion mainly by rise in the systolic pressure, while the diastolic pressure remained more or less unchanged. The noradrenaline produced a small diastolic and a more pronounced systolic increase, while the isopropylnoradrenaline produced only a slight systolic rise and a slight diastolic fall.

The *mean PCV pressure* (Fig. 3) showed a pattern similar to that of the mean pulmonary artery pressure, with rise during the adrenaline and noradrenaline infusions and a tendency to fall during the isopropylnoradrenaline infusion.

The *cardiac output* (Fig. 4) was 4.6 and 4.5 l/min 30 and 40 min after the insertion of the

catheters, indicating, as did the unchanged atrial rate, that the patients were in a steady state at the onset of the infusion. The cardiac output rose markedly during the adrenaline infusion, whereas the noradrenaline produced no change. The isopropylnoradrenaline also produced a rise in cardiac output but less pronounced than the adrenaline.

The *stroke volume* (Fig. 4) displayed a pattern similar to the cardiac output.

The *appearance time* was shortened by adrenaline from 12 to 9 sec, and by isopropylnoradrenaline from 13 to 9 sec, while noradrenaline produced no change. The *central blood volume* was not changed significantly by any of the drugs.

As expected, the *systemic vascular resistance* was increased by the noradrenaline, from 1 918 dyn sec cm^{-5} at rest to 2 378 after 8 min of noradrenaline infusion at a rate of 7.5 μg/min. The other two drugs produced a decrease of the systemic vascular resistance, the corresponding values for adrenaline being 1 749 and 1 039, respectively, and for isopropylnoradrenaline 1 918 and 1 045 dyn sec cm^{-5}.

The *pulmonary vascular resistance* (PVR) showed an initial rise during the adrenaline infusion—from 169 dyn sec cm^{-5} at rest to 226 after 8 min at 2.5 μg/min—but later fell again—to 142 after 8 min at 7.5 μg/min. The few isopropylnoradrenaline values do not indicate such a trend; the PVR remained more or less unchanged. The noradrenaline also behaved differently compared with the adrenaline: noradrenaline infusion produced a steady increase of the PVR from 151 dyn sec cm^{-5} at rest to 178 after 8 min at a rate of 2.5 μg/min and 218 at the highest concentration, 7.5 μg/min.

The *left ventricular minute work* was increased by adrenaline from 5.7 kgm/min at rest to 8.2 at the highest infusion rate. The isopropylnoradrenaline also raised the minute work but to a smaller extent, from 6.0 to 7.1. Corresponding values for noradrenaline were 7.0 and 6.7 kgm/min, respectively. As the pulse rate was constant, the *left ventricular stroke work* followed the same pattern as the minute work.

Patients with a Complete Heart block without a Pacemaker

Two of the patients, cases 1 and 3, were also examined in the same way with an infusion of catecholamines in varying concentrations, but in equimolar dosage, before they had an artificial pacemaker implanted.

The *atrial rate* showed a similar response before and after pacemaker implantation. The *ventricular rate* was increased by adrenaline from 33/min at rest to 46/min after 8 min infusion at the highest concentration. Corresponding values for isopropylnoradrenaline were 33 and 47 respectively. In one patient, case 1, the adrenaline infusion was raised from 7.5 to 13 μg/min; this resulted in no further increase of the ventricular rate, while a rise in the isopropylnoradrenaline infusion rate from 2.5 to 3.85 μg/min produced a further increase of the ventricular rate from 47 to 54/min.

The *mean aortic pressure* responded in a similar way before and after pacemaker implantation, as did the *mean pulmonary artery pressure* and the *mean PVC pressure*, although before pacemaker implantation the rise in mean pulmonary artery pressure was less pronounced during the noradrenaline infusion, and the mean aortic pressure showed an increase during the adrenaline infusion.

The *cardiac output* rose during the adrenaline infusion before pacemaker implantation from 4.3 l/min at rest to 5.8 at the highest infusion rate (7.5 μg/min). Corresponding values after pacemaker implantation were 4.6 and 6.7, respectively. Corresponding values during isopropylnoradrenaline infusion in one patient, case 1, were before pacemaker implantation 3.8 and 4.9 l/min, and after 3.5 and 4.7. Noradrenaline produced a similar response both before and after the pacemaker implantation.

The *stroke volume* was higher before pacemaker implantation than after. Noradrenaline produced no change in the stroke volume; adrenaline infusion, however, increased the stroke volume after pacemaker implantation from 69 to 104 ml at the highest infusion rate. Before the implantation the stroke volume was high (131 ml) and was not further increased (128 ml) by the adrenaline at the highest infusion rate. Isopropylnoradrenaline elicited a response similar to that of adrenaline.

The changes in *systemic* and *pulmonary vascular resistance* seemed to be less pronounced before pacemaker implantation than after, but the changes were not conclusive.

DISCUSSION

Despite the similar chemical structure of the three catecholamines used in this study—adrenaline, noradrenaline and isopropylnoradrenaline—their hemodynamic effects on the organism differ. Comparative studies have demonstrated important species differences, and data obtained with various experimental preparations do not permit direct transfer to man (9). The same drug has more than one effect. The addition of a drug such as adrenaline to a cardiac muscle preparation is followed by a complex series of events affecting both metabolism and mechanical performance. The events resulting in the increased force of contraction of the rat heart precede those associated with glycogenolysis, thus demonstrating a clear dissociation between the two effects (12).

This multiple response of a drug can probably explain some of the contradictory reports in the literature on the effect of drugs belonging to this group. The effects of noradrenaline on the cardiac output, for example, have been reported as a rise, a fall or no change at all (7).

The elevating effect of noradrenaline on blood pressure produces a reflex vagal bradycardia of varying intensity which explains some of the contradictory reports on the effect of noradrenaline on cardiac output. The fixed ventricular rate in the present study prohibits this reflex bradycardia secondary to the blood pressure rise.

There were marked differences in the hemodynamic response to the three drugs. Noradrenaline produced mainly a pressor effect, with no change in atrial or before pacemaker implantation, *ventricular rate. The adrenaline effect,* on the other hand, produced no blood pressure rise in the systemic circulation but there was a pronounced positive chronotropic and inotropic response, with an increase of atrial—and before pacemaker implantation ventricular—rate and an increase of the cardiac output. The isopropylnoradrenaline effect was similar to that of adrenaline, but there was no rise of the PCV and pulmonary artery pressures, which occurred during both the adrenaline and the noradrenaline infusions, and the positive chronotropic effect of isopropylnoradrenaline seemed to be more pronounced than that of adrenaline. This was apparent both in the patients with a fixed rate pacemaker in whom the atrial rate rose to a very high level (Fig. 2) and in one of the patients before pacemaker implantation, with increasing doses of the ventricular rate leveled off during the adrenaline infusion, while there was a further rise with increasing isopropylnoradrenaline concentration. This confirms the clinical impression of isopropylnoradrenaline as the drug of choice in many patients with bradyarrhythmias and Adams-Stokes attacks.

The present results for the noradrenaline infusion differ from those published by Judge et al. (7). These authors reported an increase of the cardiac output during an intravenous infusion of noradrenaline at a concentration of 4 to 8 μg/min. They determined the cardiac output when an increase in systolic aortic pressure of 30 to 70 mmHg had occurred. The noradrenaline concentration reported by Judge et al. is similar to that in the present study, but we did not obtain a systolic aortic pressure increase higher than 25 mmHg, which may partly explain the different results. The effect of isopropylnoradrenaline fits in well with the results reported by Stack et al. (10), Benchimol et al. (2) and Ino et al. (8).

There was a marked difference between the three drugs in their effect on the pulmonary vascular resistance (PVR). Noradrenaline increased the PVR, as did adrenaline initially. This similar result is of interest in relation to the different results of these two drugs on the systemic circulation, with noradrenaline raising the pressure and keeping the *flow* unchanged and adrenaline raising the flow and keeping the pressure at an unchanged or lowered level. This indicates that the systemic and the pulmonary circulations can be regulated separately.

The increase in pulmonary pressure elicited by noradrenaline might be explained by a constriction of the pulmonary vessels, resulting in an increased stiffness of the vessels (5). The increase in PCV pressure with both adrenaline and noradrenaline is remarkable. It cannot be explained by increased flow, because noradrenaline kept the cardiac output unchanged and isopropylnoradrenaline increased the flow but not the PCV pressure, rather the reverse. Constriction of the pulmonary veins is a possible explanation.

When comparing the hemodynamic response to the three drugs before and after pacemaker implantation in two of the patients, no major differences were found despite the constant en-

tricular rate after the pacemaker implantation. There were some minor differences, however The cardiac output increased more after pacemaker implantation. Before implantation adrenaline produced an increase of the aortic pressure while noradrenaline caused a less pronounced increase of the pulmonary artery pressure

The hemodynamic response was remarkably consistent in the patients in the present study when taking into consideration the different conditions of the myocardium and circulation. This response conforms with the results reported by Dodge et al. (4). These authors studied the cardiovascular effects of isopropylnoradrenaline administered intravenously to three subjects with essentially normal heart function and 18 subjects with congestive heart failure of varying etiologies. Their observations indicated a similar response to the drug in both groups. The hemodynamic response to drugs can of course be changed if the clinical condition is seriously impaired. Brown et al. (3) found some variations in the response to noradrenaline metaraminol and isopropylnoradrenaline in patients in a state of shock. It was postulated that these variations may be related to the severity of the shock.

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SINGLE CORONARY ARTERY

A Report of Three Cases

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Abstract. Three cases of single coronary artery are reported. All had bicuspid aortic valves and one case also ventricular septal defect. A 24-year-old woman had suffered from angina pectoris from the age of 16. Over the last years electrocardiographic signs of progressive myocardial ischemia and an additional right bundle branch block have developed. Angiography has shown the presence of single left coronary artery and bicuspid aortic valves. A 65-year-old woman with aortic stenosis and insufficiency has single right coronary artery and bicuspid aortic valves. The ECG reveals the presence of left bundle branch block. A man aged 34 years with single left coronary artery, bicuspid aortic valves and ventricular septal defect had subacute bacterial endocarditis with rapid development of aortic insufficiency. He died from intractable heart failure. Emphasis is laid upon the recognition of single coronary artery as the potential cause of severe heart disease such as subacute bacterial endocarditis, aortic valvular disease and premature myocardial ischemia.

Single coronary artery means the congenital absence of one coronary artery so that the entire heart is supplied by one coronary artery alone.

The anomaly was formerly regarded as clinically insignificant (5, 6, 9), a view which can hardly be maintained. On the contrary there are at least three reasons to consider it a potential cause of adult heart disease.

Firstly it is often associated with bicuspid aortic valves (14) and may correspondingly give rise to aortic stenosis and incompetence. Secondly the frequent association with other congenital heart defects (1, 2, 9, 10) may cause subacute bacterial endocarditis. In fact the 50% death rate recorded in subjects below the age of 20 years is due to the presence of the varied series of such congenital defects (9). Thirdly obstructive disease in a single coronary artery may produce myocardial necrosis and ischemia and possibly also sudden death from cardiac arrhythmias (2, 3, 6).

The coronary artery anomaly is by no means innocent, which is proved by the observation of a definitely reduced life expectancy of its carriers (1).

Single coronary artery is rare. Until now only 85 cases have been reported (1, 9, 12). A majority have been diagnosed at autopsy or during heart surgery (2, 3, 4, 5, 6, 8, 9). The advent of coronary angiography during the last decade has made it possible to recognize the condition in live subjects and to institute appropriative treatment. It has consequently become important to disclose single coronary artery as the accessory or main cause of heart disease.

This report presents the history and the clinical findings of three adult subjects with single coronary artery. The hazards associated with the anomaly are fairly well illustrated.

CASE REPORTS

Case 1

A woman born in 1944 had from childhood complained of exertional dyspnea and muscular fatigue, and at the age of 16 typical angina pectoris occurred. In the following year she went through an uneventful pregnancy but the heart was found to be enlarged.

In 1965, 21 years of age, she was admitted to our Medical Department for evaluation of premature angina pectoris. Examination revealed the presence of funnel chest. The apex beat was felt 9 cm from the sternal midline. A systolic murmur was audible over the whole precordial area with the maximal strength of grade 4 over the 2nd left interspace. The second pulmonic sound was slightly accentuated, but not split. The second aortic sound was normal. No signs of congestive failure could be demonstrated. The ECG (Fig. 1) showed atypical left

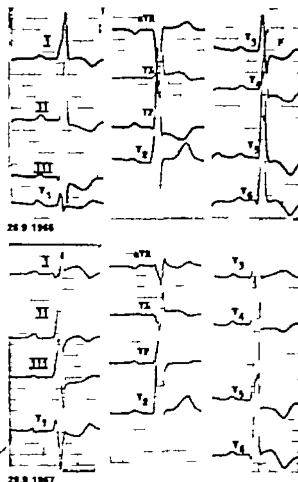


Fig. 1. ECGs of case 1 from 1965 and 1967

retardation and left ventricular systolic overload. A chest film revealed funnel chest with left displacement of an otherwise normal-sized heart silhouette but distinct biventricular hypertrophy was visible.

Both right and left heart catheterization showed normal pressures and oxygen saturations at all levels. Cineangiography of the left ventricle likewise revealed normal conditions, except for the presence of bicuspid but competent aortic valves (Fig. 2). Subsequent coronary angiography made possible the detection of a single coronary artery (Fig. 2 *b*), namely the left one, which departed from a normally situated ostium in the left coronary sinus. At a distance of about 2 cm from the ostium it gave off the right coronary artery traversing to the right between the aortic and pulmonary roots and dividing in grossly normal manner. The type of anomaly present was therefore of type I according to the classification made by Smith (15).

The patient was readmitted in 1967 due to increasing severity of the angina. Despite the absence of any episode indicative of myocardial infarction, the ECG showed changes consistent with a recent anterior infarction (Fig. 1). No support for such diagnosis could be obtained by means of biochemical or hematological studies.

In 1968 the condition of the patient was unaltered, but the ECG showed progressive signs of recent anterolateral myocardial infarction, and in addition right bundle branch block had now appeared (Fig. 3).

At the last check-up in 1969 she still complained of a severe angina pectoris, and the ECG showed even more distinct signs of myocardial ischemia and necrosis.

This case report demonstrates that a single *left* coronary artery although giving off an apparently normal right artery with normal distribution, is the cause of heart symptoms from childhood and of severe, *progressive myocardial ischemia* in adult age. Notable is also the development of a *right bundle branch block* and the simultaneous presence of *bicuspid* but competent aortic valves.

Case 2

A 65-year-old woman was admitted for hemodynamic investigation of an aortic failure. For the last 3 years she

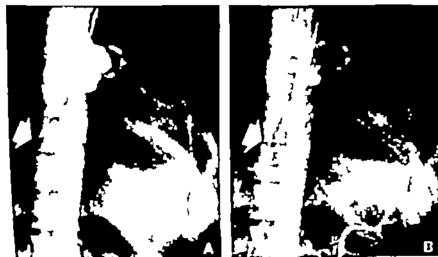


Fig. 2. (*A*) Bicuspid aortic valves and (*B*) single left coronary artery found in case 1. White arrow indicates right coronary artery, large black arrow indicates circumflex and small black arrow anterior descending arteries.

had been troubled by angina pectoris, tachycardia and syncopes on effort. B.P. was 160/100 mmHg. Auscultation revealed typical signs of aortic stenosis and insufficiency. A left bundle branch block was seen on the ECG, while the X-ray examination showed a normal heart size, but definite left ventricular hypertrophy. By left heart catheterization a pressure gradient across the aortic valves of 64 mmHg was found. Cineangiography showed the presence of bicuspid aortic valves with stenosis and incompetence and mitral insufficiency. Finally a single right coronary artery was detected by means of coronary angiography (Fig. 4). The right coronary artery emerged from its usual position in the right aortic sinus and immediately gave off a main branch, which functionally was the left coronary artery. This divided into the anterior descending and the circumflex branches in a normal manner. A little doubt arose as to whether the left coronary artery originated from a separate ostium in the right aortic sinus, but study of the film confirmed the existence of a single ostium. The patient was a candidate for surgery with insertion of a ball valve prosthesis at the aortic orifice.

This case again illustrates the association of a single coronary artery with *bicuspid aortic valves*, which are the seat of both stenosis and incompetence. Moreover the single *right* coronary artery is present at the same time as a *left bundle branch block*.



Fig. 4. Cineangiogram of case 2 illustrating bicuspid aortic valves and single right coronary artery. Arrow indication as in Fig. 2.



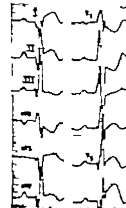


Fig. 3. ECGs of case 1 from 1968 and 1969 with definitive signs of progressive myocardial ischemia.

Case 3

A man aged 34 years was admitted in 1967 due to recent subacute bacterial endocarditis with rapid development of aortic insufficiency and concomitant congestive failure. When at the age of 10 a systolic cardiac murmur interpreted as due to a ventricular septal defect had been heard. He had been in excellent health until 6 months prior to the admission. Then he had noted a small but distinct exertional dyspnea. Three months later he became acutely ill with fever and malaise. He was referred to the regional hospital, where numerous blood cultures were negative. Despite this he received vigorous treatment for subacute bacterial endocarditis. He did not respond to penicillin or tetracycline, but ultimately became afebrile by means of cephaloridine and streptomycin. In the meantime his heart size increased, a rapidly increasing aortic insufficiency was noted and a marked congestive failure developed.

On arrival in our department he was cyanotic, edematous, had severe pulmonary congestion and an enlarged, pulsating liver. The ECG showed left ventricular systolic overload, while the chest film revealed a large heart with a dilated left ventricle. Subsequent right heart catheterization demonstrated the presence of a ventricular septal defect with a left-to-right shunt of 42% of the pulmonary blood flow and a right-to-left shunt of 17% of the systemic blood flow. A tricuspid insufficiency was also re-

gistered. At cineangiography the aortic valves were found to be bicuspid and markedly incompetent, and a mitral insufficiency was also observed. Furthermore the ascending aorta was the seat of conspicuous aneurysm. Coronary angiography revealed the presence of single left coronary artery. This originated from the normal site in the left aortic sinus and gave off a small branch running over to the right side of the heart. Unfortunately the patient was critically ill, so that further examinations including recordings of left heart pressures had to be omitted.

He was referred to the surgical department for insertion of a prosthetic aortic ball valve on vital indication. A thoracotomy was done, but the ascending aorta was so dilated and thin-walled that repair of the aortic valves could not be performed.

The patient was sent to the regional hospital for further treatment of the congestive failure and died two months later. No autopsy was done.

This case illustrates the simultaneous presence of a *single coronary artery* a *ventricular septal defect* and *bicuspid aortic valves*, which obviously became the seat of a *subacute bacterial endocarditis*

DISCUSSION

The three cases reported reflect the essential consequences of the presence of a single coronary artery

Firstly the frequent association with bicuspid aortic valves is to be emphasized (14) because the latter at present is a major etiological factor in aortic stenosis and insufficiency (16). Moreover the liability of such cusps to become the seat of subacute bacterial endocarditis has been known for a long time (13). This serious complication has been frequently observed in subjects with a single coronary artery (2, 14).

Secondly the myocardial blood supply in single coronary artery has to be considered. The cases in this report all showed a grossly normal distribution of the coronary arteries and their branches, except for the anomalous origin of one of the main arteries. Thus the coronary circulation should presumably be normal. Although this may be the case (5 6, 9), there is still reason to regard single coronary artery as a potential cause of myocardial ischemia. Until now nine cases of myocardial ischemia and necrosis have been reported, seven men and two women (1 2, 6, 11 14 17). Seven had single right and two single left coronary artery The present report adds one woman with single left coronary artery Her case history is notable for the fact that cardiac complaints were present from childhood and that premature angina pectoris was followed by progressive myocardial ischemia. It is reasonable to believe that the anomalously originating vessels, with their increased length and many bends, are especially prone to become the seat of obstructive lesions. Occlusive thrombosis and atherosclerosis have been found at autopsy in many cases with single coronary artery and support this view (1 2, 3 14).

Thirdly a new aspect of single coronary artery is revealed by this report, namely its possible effect upon the conductive system of the heart. In case 1 a left coronary artery was associated with the simultaneous development of myocardial ischemia and a right bundle branch block. In case 2 a right coronary artery was present together with a left bundle branch block. The coincidence of a single coronary artery and a contralateral bundle branch block is hardly accidental. The events noted in case 1 moreover make it likely that disturbances of the myocardial blood supply are the cause. A recent communication (7) has drawn attention to the existence of an abnormally short main stem of the left coronary artery in left bundle branch block. In case 2 of this report the respective main stem was, on the contrary abnormally long.

It is unknown to what extent single coronary artery by affecting the conductive system, predisposes to arrhythmias and sudden death, but several reported cases indicate that such a possibility is real (2, 3). Single coronary artery is of rare occurrence, and it should be noted that our three cases were found among 1 056 coronary angiographies. *The general incidence is reflected* by the report of Alexander and Griffith (2), who detected seven cases in a series of 18 950 autopsies. Also among the coronary artery anomalies the single artery is of modest frequency as shown by Ogden (12). In his series of 224 cases only 10 cases were single coronary artery

Despite its rare occurrence single coronary artery is important to recognize. Firstly because it is the potential cause of severe heart disease such as subacute bacterial endocarditis, aortic valvular disease, myocardial ischemia and possibly bundle branch block and rhythm disturbances. Secondly because its recognition widens the scope of heart diagnosis, especially with regard to the etiology of myocardial ischemia, which for so long a time

has been nearly uniformly ascribed to the presence of lipid metabolic disorders.

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Acta med. scand. Vol. 189 pp. 415–422, 1971

STUDIES IN SUBJECTS WITH POSITIVE POSTPRANDIAL CLINISTIX® TEST

III. *Special Studies and Follow-up of Cases with Borderline Glucose Tolerance*

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Abstract. Twohundred and thirty-one subjects with borderline glucose tolerance, detected during a diabetes survey performed in Malmöhus County 1962–1965, have been followed up for 3–5 years. In the newly discovered state selected cases were studied with determinations of serum-insulin-like activity (SILA) in the fasting state and during oral glucose tolerance tests (OGTT), and with determinations of plasma free fatty acids (FFA), fasting and during a short exercise test. It was found that the SILA values of the borderline cases did not differ from those of normal subjects. In the fasting state the plasma FFA level of the borderline cases was somewhat elevated, but during the exercise test no difference was noticed in comparison with normal subjects. Intravenous glucose tolerance tests (IGTT) were performed in the subjects in groups II–IV and in a control group. The K-values in the borderline groups were significantly lower than that of the control group. The borderline cases were separated into four groups: group I was given no instructions at all, group II dietary instructions, group III diet and tolbutamide 1.5 g daily and group IV diet and placebo tablets. In none of the groups did any individual pass into a state of clinical overt diabetes during the observation period, and no deterioration of the carbohydrate tolerance was observed as judged by repeated OGTTs. It is concluded that the progress of glucose intolerance, if any, is slow and that further observation of the borderline cases is necessary to reveal whether such subjects are candidates for clinical diabetes.

As stated by Wilson and Jungner (40), the object of screening for disease is to discover those among the apparently well who are in fact suffering from disease. Such subjects will obviously show less pronounced clinical and laboratory signs of disease. They should not be informed that they are sick if this cannot be proven and if the information does not lead to therapy. Obviously we are here entering a no-man's-land, hoping to discover diseases at a very early stage when treatment should prevent the establishment of manifest chronic disease and its secondary complications.

Individuals with borderline carbohydrate tolerance have been assumed to represent an early phase of diabetes and should, if the theory were true, be the target of diabetes screening programs, in addition to those, of course, with manifest undiscovered disease. The present report deals with a borderline group which has been followed by clinical examination and repeated carbohydrate tolerance tests during an observation period of two to five years.

Lately another justification for the search for individuals with moderate hyperglycemia, namely its possible though hitherto unproven relation to cardiovascular disease, has been brought up (15, 21, 37). The prevalence of cardiovascular disease in the borderline group under study will be the object of a separate report (9).

Borderline cases are defined in the present study as those with an oral glucose tolerance curve above the upper border for a normal group of subjects of the same age, selected from the same population, and below the border of a diabetic group detected during the survey. Details concerning the survey have already been presented (23). The diabetics diagnosed during the survey and a follow-up study of them, will be presented separately (8).

MATERIAL AND METHODS

The clinical material consisted of 578 subjects. Borderline carbohydrate tolerance was considered present when all ten capillary blood glucose measurements during the oral

Table I

	Males		Females		
Group	No.	Age	No.	Age	Treatment given
I	101	54 ±11.8	6	52 ±11.3	None
II	36	54 ±13.3	6	57.8± 9.8	Diet
III	47	54 4±12.8	7	45 6±17 0	Diet + 1.5 g tolbutamide
IV	41	57 9±12.4	6	61.8±10 0	Diet + placebo
Controls	33	43 5±14.6	25	43.2±12.8	

glucose tolerance test (OGTT) were above the mean −2 S.D for the corresponding normal age and sex group. The upper demarcation line was represented by the mean −3 S.D. *Diabetes was diagnosed when all raw blood* glucose values were above the mean +3 S.D for the corresponding normal group A few values only above the mean +3 S.D. would then still place the individual curve in the borderline group. The results for the normal group in order to establish the criteria of the OGTT have been published earlier (32).

From the original material with borderline carbohydrate tolerance 251 subjects were selected at random for follow-up 231 of whom have hitherto completed the study *Group I* consisted of 107 individuals who were informed that they did not have diabetes. They were examined at the start of the study and reexamined 2 5 years later

124 subjects were treated. They were originally informed that they possibly were candidates for manifest diabetes. No serious dietary restrictions were presented, but it was suggested that they should limit their intake of carbohydrates and fat. Those who were overweight were advised to reduce their caloric intake.

The treated subjects were divided at random into three groups.

group II 36 subjects, only given dietary advice

group III 47 subjects, in addition treated with 1.5 g tolbutamide (Ineco Co., Stockholm) per day

group IV 41 subjects, given the same dietary instructions and placebo (Ineco Co., Stockholm).

The active drug and the placebo were labelled with numbers and supplied through the Hospital Pharmacy Age and sex of patients in the groups are given in Table I.

The borderline group was dominated by males. The age distribution in the four groups of men was comparable, but in the small number of females the variation was considerable. All subjects were examined clinically and by OGTTs at 3-month intervals during the first eighteen months, and from then on every sixth month. At the fifth examination an intravenous glucose tolerance test (IGTT) was performed instead of the OGTT

Fifty-eight subjects without heredity for diabetes, selected at random from the population, were subjected to IGTT to serve as controls. Age and sex of the control subjects are presented in Table I.

Before starting treatment special studies such as determinations of the serum-insulin-like activity (SILA), fasting and during OGTT determination of free fatty acids (FFA), fasting, during and after a short period of exercise were conducted in selected cases.

The participants were instructed to take bread with every meal for three days before the OGTT as recommended by Conn (12). The blood glucose was determined according to Marks (27) as standardized by Scherstén (31).

The OGTT was performed in the fasting state at 9 a.m. A dose of 30 g of glucose/m² body surface area was served in 10% solution. Capillary blood glucose was determined in the fasting state and after 15 30, 45 60, 75 90, 120, 150 and 180 min. The subjects were resting during the test in a half-recumbent position in comfortable chairs. The urine was tested for glucose by Clinistix® after one, two and three hours. This was the only movement out of the chair permitted. Smoking was not allowed. No extra fluid intake was permitted during the test.

The IGTT was performed under the same standardized conditions with the subjects in supine position. An amount of 100 ml of 30% glucose solution was injected intravenously within 4 min. When half the amount had been injected, a stop watch marking zero time was started. The blood glucose concentration was determined at 10, 20, 30 40, 50, 60, 75 and 90 min later The blood glucose concentrations were plotted against time on a semilogarithmic diagram. The K-values were estimated from the slope of the curve obtained between 25 and 60 min.

In some of the subjects the SILA levels were determined in the fasting state and during OGTT and the levels were compared with those of normal subjects and diabetics earlier studied in the same way (23)

Other subjects selected from the borderline group were studied by a physical exercise test performed on a bicycle ergometer for 10 min. Before, during and after exercise, blood samples were drawn through an indwelling arterial catheter and the plasma FFA concentrations were de

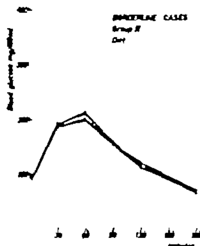


Fig 1 Mean blood glucose curves during four OGTT in group II.

termined. Details concerning the performance of the test have been published earlier (7). The borderline cases were compared with control subjects and newly diagnosed juvenile diabetics, studied under identical conditions and earlier presented (7). FFA was titrated according to Trout et al. (36) and the SILA was estimated according to Lyngsøe (24).

For the statistical evaluation of the OGTT of groups II, III and IV analyses of variance were performed (34). As regards group I the two OGTT were compared by means of Student t-test (34), which was also used when the results of the IGTT of groups II, III and IV were compared with that of the control group and when the fasting SILA levels of the different groups were considered. Wilcoxon rank sum test (39) was used when small groups of subjects were compared, as in the SILA study during OGTT and the FFA study during exercise.

RESULTS

During the first year of observation four OGTTs were performed in subjects belonging to groups II, III and IV. The results of the tests are summarized in Figs. 1, 2 and 3, respectively. As seen from the figures, no great differences were found. The individual blood glucose concentrations during the tests have been evaluated by means of variance analysis (34). The F-values for the blood glucose concentrations at different times during the OGTT for the separate groups are summarized in Table II. In group II there is a probably significant lowering of the values at 60 min, but no other variations. This group had only received dietary instructions.

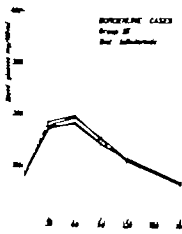


Fig. 2. Mean blood glucose curves during four OGTT in group III.

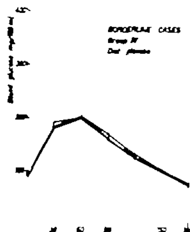


Fig. 3. Mean blood glucose curves during four OGTT in group IV.

Group III, which had been treated with tolbutamide and given dietary advice, showed a significant decrease of the blood glucose values ($p < 0.01$) at 30 and 60 min, fitting with an increased insulin release through tolbutamide as described by Cerasi and Luft (11). Fasting and at 90 min the decrease was only probably significant ($p < 0.05$). In the placebo-treated group IV the only change was a probably significant decline at 30 min. However, all the recorded variations were small, and all the mean blood glucose curves in the group classed as borderline carbohydrate tolerance according to our criteria remained unchanged, which is still true after five years' follow-up.

Results of OGTT in the untreated group I are illustrated in Fig. 4, which shows the two mean blood glucose concentrations, determined initially and at the end of the follow-up period. No significant difference was found between the first and the second test, suggesting that no impairment of carbohydrate tolerance had taken place. None of the studied subjects in any of the groups has shown symptoms of clinical overt diabetes during the follow-up period, and none has developed a diabetic glucose tolerance as judged from the OGTTs.

The K-values of the control subjects studied with the IGTT are presented in Fig. 5. The mean value of the group is 1.61 ± 0.63 and the median value 1.40. The mean K-values and the S.D. of →

Table II. *F-values of groups II–IV determined by variance analysis of blood glucose*
Concentrations at different times during 4 OGTTs. Significance levels given within brackets

Group	Fasting	30 min	60 min	90 min	120 min	180 min
II (n = 36)	0.324	0.524	3.339 ($p < 0.05$)	0.618	1.644	2.069
III (n = 47)	3.748 ($p < 0.05$)	4.392 ($p < 0.01$)	6.966 ($p < 0.01$)	3.341 ($p < 0.05$)	0.494	1.418
IV (n = 41)	0.979	3.475 ($p < 0.05$)	0.686	0.980	0.964	2.519

groups II, III and IV are given in Table III. The K-value in the borderline cases was significantly lower than that of the control group.

Fig. 6 shows the mean fasting SILA levels of the borderline cases. In comparison with the mean fasting SILA levels of one group of control subjects and one group of diabetics, no differences were found. The SILA values during the OGTT are graphically presented in Fig. 7. No difference was noticed between the borderline cases and the control subjects, whereas the diabetics clearly differed from the other groups.

The plasma FFA concentrations of the borderline cases before, during and after exercise are presented in Fig. 8. For comparison, the FFA curves for one group of control subjects and one group of juvenile, non-insulin treated diabetics are shown. Before exercise the FFA values are probably significantly higher in the borderline group than in the control group ($p < 0.05$). During and after exercise no such difference is found. On all occasions the FFA values of the borderline group are significantly lower than those observed in the diabetic group ($p < 0.01$).

The results may be summarized as follows. Subjects with borderline carbohydrate tolerance, as judged from the oral glucose tolerance test, also showed borderline tolerance in the intravenous glucose tolerance test. Their SILA response to oral glucose was, however, normal, and their fasting SILA did not deviate from levels observed in normal subjects. The fasting free fatty acid level again suggested that they represent an intermediate group between normals and diabetics.

During an observation period of 2–5 years no case of manifest diabetes was observed in 231 subjects with borderline carbohydrate tolerance. Furthermore, carbohydrate tolerance remained constant and independent of dietary restrictions or treatment with tolbutamide.



Fig. 4. Mean and S.D. of the blood glucose concentrations during two OGTTs in group I, performed initially and after 2–5 years' follow-up.

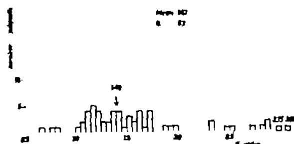


Fig. 5. K-values of the IGTT performed on 58 control subjects.

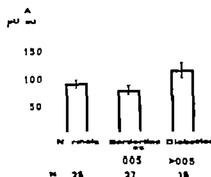


Fig 6. SILA levels (mean ± mean range) in the fasting state of 27 borderline cases, compared with normals and diabetics.

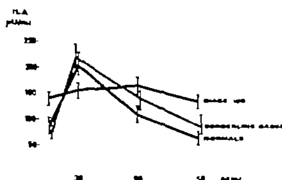


Fig 7. SILA levels (mean ± mean range) during OGTT of 8 borderline cases, compared with normals and diabetics.

DISCUSSION

Borderline glucose tolerance is of interest with respect both to the possibility of an early indication of diabetes and to its relation to cardiovascular disease (15 21 37) The present report concerns aspects related to diabetes or more strictly to the glucose tolerance. The same subjects are now being studied from a cardiovascular point of view (9).

Table III. *Intravenous glucose tolerance tests*

Group	K-value	Compared with the control group, *p*
II	1.07±0.33	0.001
III	1.11±0.49	0.001
IV	1.05±0.28	0.001

The borderline cases of the present study were diagnosed by an oral glucose tolerance test, performed under strictly standardized conditions, employing criteria established in an age and sex matched control group selected at random from the same population (32). Many other tests have been used to diagnose borderline or latent diabetics (2, 3 20 26) but we agree with Alexander's (1) opinion that the oral glucose tolerance test, if performed properly remains the cornerstone in the diagnosis of diabetes

However the borderline cases of the present study could also be distinguished from group of control subjects by the intravenous glucose tolerance test. Furthermore, the free fatty acid level determined in the fasting state was higher than in controls and lower than in patients with diabetes. The plasma FFA concentration during a cortisone-OGTT has been used to diagnose individuals with early latent diabetes (33), and Hales (18) has reported a case of juvenile diabetes with

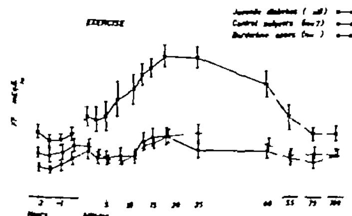


Fig 8. Plasma FFA concentrations (mean ± S.E.M.) before, during and after 19 min exercise test of 6 borderline cases, compared with non-insulin treated, juvenile diabetics and control subjects.

elevated levels of plasma FFA and plasma glycerol four years before the onset of clinical diabetes. At that time the patient showed only a minor abnormality in the oral glucose tolerance and was asymptomatic. In the present study the plasma FFA curve was followed during a 10-min exercise test. The response to exercise was identical with findings made in a control group. Thus the extraordinary exercise-induced lipid mobilization observed in diabetics (7 10) was not found

The SILA concentration in the borderline cases in the fasting state and SILA response during the OGTT did not differ from that found in control subjects. The serum immunoreactive insulin level in the early stage of latent diabetes has been reported to be elevated (3 19 28) but on the other hand it has been pointed out that the elevation is primarily correlated to overweight (4 19). Reports on the insulin level measured with the fat-pad technique in borderline cases have, as far as we know not been published.

Prospective studies on subjects with minor abnormalities of glucose tolerance have been made by several authors (6 14 22, 28 29 35 38). In the Bedford study (6 20), borderline carbohydrate tolerance was identified by showing a lary blood glucose between 120 and 200 mg/100 ml two hours after 50 g glucose by mouth. A controlled follow-up was organized so that half the subjects received 500 mg tolbutamide daily the other half being given 3 mg of the drug per day Half of each group were given dietary instructions. The follow-up consisted of visits at 6 months intervals, at which time a questionnaire was answered, blood pressure recorded, pulses palpated, and fasting blood glucose measured. The OGTT was repeated each time. The mean blood glucose in the treatment and control groups showed, after five years, no systematic difference.

Engelhardt and Veccio (14) have reported a study on asymptomatic, latent diabetics with a fasting blood glucose below 11? mg/100 ml one hour values during OGTT exceeding 170, and two-hour values above 1?0/100 ml. They followed their patients for 8 to 24 months. Half the subjects were given tolbutamide, 1 g daily the other half placebo tablets. The patients were controlled every third month with an OGTT A probably significant decrease of the one-hour values and a significant decrease of the half-hour values during OGTT were found in the tolbutamide-treated group. Feldman and Fitterer (17) studied subjects with asymptomatic diabetes who were given either tolbutamide, phenformin or placebo. The changes in glucose tolerance after one year showed a return towards a normal glucose tolerance in those receiving tolbutamide. The placebo group showed similar improvement. With phenformin no improvement was observed. Tolbutamide did not affect the insulin response to glucose. Another group of 219 subjects with definite, and 39 with probable, diabetes showed improved glucose tolerance in 63.3% of those treated with tolbutamide in 26 7% of those given placebo, and in 10.0% of the phenformin group. The abnormality increased in 26.9% of those treated with tolbutamide, in 34.6% in the placebo group, and in 38.5% in the phenformin group.

In the present study we noticed a minor decrease in the blood glucose levels during OGTT in the tolbutamide group (group III) in comparison with the other groups. Our results thus agreed with those of Engelhardt and Veccio (14). However the differences were small, and in none of the studies have any of the subjects developed diabetic symptoms. In calculations based on the Birmingham studies, Anderson (3) arrived at the conclusion that in the 40–50 age group diabetes might remain unrecognized for 10 to 12 years.

The present studies were all performed fasting in the morning at 9 a.m. The times at which the patient left home and arrived at the laboratory were recorded to make sure that the time factor would not influence the reproducibility of repeated glucose tolerance tests. It is known that the response to glucose is influenced by the time of day (5) Another point which may have contributed to satisfactory reproducibility is that the glucose solution was always given as a 10% solution. Occasionally 100 g of glucose is given in a total volume of 300 ml, and even concentrations as high as 50% have been used (25). In our experience the use of highly concentrated solutions is frequently followed by nausea and may affect the emptying of the stomach.

Patients with borderline glucose tolerance have often been looked upon as candidates for clinical diabetes. However hitherto all studies, the present one included, have failed to prove definitely that a minor glucose intolerance necessarily re-

presents an early stage of diabetes. Apparently the progress of the glucose intolerance, if any is extremely slow and further observation is necessary to solve the problem.

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EFFECT OF LB 46, A NEW BETA ADRENERGIC ANTAGONIST IN HYPERTHYROIDISM

Heart Rate and Blood Pressure at Rest and during Exercise

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Abstract. The effect of oral administration of a single dose of 5 mg (6 patients) and 10 mg (6 patients) of LB 46 (Sandoz Ltd., Basel), a new β-adrenergic antagonist, or placebo (3 patients), on the heart rate and blood pressure at rest and during exercise on a bicycle ergometer has been studied in patients with untreated hyperthyroidism. The two doses had essentially similar effects and decreased the heart rate by about 20%, both at rest and during work. There was a marked increase in the calculated physical working capacity at 170 heart beats/min (PWC_{170}) but no significant change in the actual physical working capacity. There was a slight but statistically insignificant decrease in blood pressure. Five mg of LB 46 was given every 8 h for 1 to 3 weeks without any other medication, and the exercise test was then repeated with essentially similar results. Two patients developed transient disturbances of cardiac rhythm during ergometry after 10 mg of LB 46, but all the patients did well during the subsequent continuous administration of the drug. *It is concluded that LB 46 is effective in decreasing tachycardia in hyperthyroidism and, mainly in this way it increases the* PWC_{170}.

Beta-adrenergic blockade is well established for the symptomatic treatment of the hyperkinetic circulation in hyperthyroidism (1–4), the rationale being the increased sympathetic drive and the increased sensitivity to circulating catecholamines in this state (3–7). It would thus appear appropriate to use agents exerting a maximal blocking effect and a relatively small intrinsic sympathomimetic effect. The drug should also have a minimal direct depressive effect on myocardial contractility. A new agent fulfilling these criteria, *d,l*-4-(2-hydroxy-3-isopropylaminopropoxy)-indol (LB 46) (5, 8), has recently been developed and is effective in smaller doses than any previously reported beta-adrenergic antagonist. A report is given on its effect on pulse rate and blood pressure at rest and during exercise, as well as on the physical working capacity (PWC_{170}) in hyperthyroid patients.

MATERIAL AND METHODS

Fifteen patients who were attending the outpatient department with unequivocal hyperthyroidism diagnosed on clinical and laboratory criteria (6) were tested by ergometry prior to medication and on a separate day 1 h after oral administration of either 5 mg (6 patients) or 10 mg (6 patients) of LB 46 or placebo (3 patients), employing a double blind test design. The mean age of the patients was 29.6 years (16–51). 12 were women and 3 men (nos. 1, 13 and 14). None of the patients had any complicating diseases affecting their respiratory or cardiovascular functions. The exercise tests were performed in a postabsorptive state in the afternoon 6 h after a light meal in the morning. After this acute test the drug was continued at 5 mg every 8 h for one to three weeks. Ergometry was then performed a third time under similar conditions 1 h after the last dose of LB 46. In order to compare the effects of a single dose (5 or 10 mg) with those of continuous administration, the results of the acute tests were compared with those obtained after continuous administration for one to three weeks. This part of the series included 10 patients, since those who received placebo in the acute test were omitted along with two other patients who did not arrive for the last exercise test, although they had taken the drug regularly. The patients received no other *medication throughout the experimental period*.

In all the tests an electrically braked bicycle ergometer (Elema-Schönander, Sweden) was used. The patient pedalled the bicycle ergometer in the supine position, and the load was increased by steps 150–300–450–600, etc. kpm/min, each load being kept up for 6 min. The heart rate was determined from the ECG, and Åstrand's steady-state criteria (9) in respect of heart rate change

Table I *The effect of LB 46 on the heart rate at rest and during work*

	Exercise (kpm/min)				
	Rest	150	300	450	600
Control values, no drug given					
Mean heart rate	108	139	148	162	159
(Range)	(72 125)	(124–158)	(99–180)	(14 180)	(113–180)
No. of pats.	15	13	15	11	7
Single dose 5 mg LB 46					
Mean heart rate control heart rate	−19.66	−30.00	−28.16	−34.75	−32.00
(Range)	(−4 −35)	(−25 −33)	(−8 −40)	(−31 −42)	(−11 – −48)
No. of pats.	6	5	6	4	4
Significance of drugs effect	$p<0.05$	$p<0.01$	$p<0.1$	$p<0.01$	$p<0.05$
Single dose 10 mg LB 46					
Mean heart rate control heart rate	−10.83	−19.16	−27.66	−31.75	—
(Range)	(+11 −20)	(+3 −42)	(+1 −47)	(−6 −47)	—
No. of pats.	6	6	6	4	
Significance of drug effect	N.S.	$p<0.05$	$p<0.05$	$p<0.05$	
Placebo					
Mean heart rate control heart rate	−6.33	−1.00	−4.66	−10.00	−0.50
(Range)	(+16 −34)	(+3 −5)	(−1 −10)	(−5 – −18)	(+4 −5)
No. of pats.	3	2	3	3	3
Significance of drug effect	N.S.	N.S.	N.S.	N.S.	N.S.
Continuous administration for one week of 5 mg LB 46 every 8 h					
Mean heart rate control heart rate	−4.16	−18.60	−22.33	−29.25	−22.60
(Range)	(+10 −15)	(+6 −28)	(+5 −45)	(+3 −44)	(+6 −47)
No. of pats.	6	5	6	4	5
Significance of drug effect	N.S.	$p<0.05$	$p<0.05$	N.S.	N.S.

N.S. = not significant

during the last 4 min of each period were applied in interpreting the records. The highest steady-state heart rate then determined the end point by which the PWC_{170} was estimated according to the method of Sjöstrand (9). The test was continued up to the limit of the subject's exercise tolerance.

RESULTS AND DISCUSSION

Table I shows that the acute administration of LB 46 clearly decreases the heart rate at rest and during exercise at all levels of work load employed. The drug generally decreased the heart rate by about 20% and no effect was seen after administration of placebo. No significant difference was seen between the two dose levels. The dispersion of the results was somewhat larger in the group receiving 10 mg, and the statistical significance thus correspondingly lower. When the exercise test was repeated on 6 patients after one

Table II *Comparison of the effects of acute and continuous administration of LB 46 on the heart rate*

	Exercise (kpm/min)				
	Rest	150	300	450	600
Mean heart rate after continuous administration (1 h following the last dose, 5 mg) – heart rate after acute administration (5 or 10 mg)	+3.60	+7.55	+2.60	+6.00	+3.75
(Range)	(+12 −12)	(+27 −1)	(+20 −9)	(+12 −9)	(+10 −1)
No. of pats.	10	9	10	6	4
Significance of the difference	N.S.	$p<0.05$	N.S.	N.S.	N.S.

Table III. *Effect of a single dose of LB 46 on PWC_{170}*

Pat. no.	PWC_{170} (kpm/min) Control value	Following LB 46	Increase
5 mg LB 46			
1	475	1 100	+625
5	500	1 180	+680
6	265	690	+425
10	465	805	+340
13	1 350	2 100	+750
14	665	1 185	+520
Mean	620	1 177	+557
Significance of drug effect			$p < 0.01$
10 mg LB 46			
2	390	1 100	+710
4	600	1 275	+675
7	235	1 070	+835
8	950	1 500	+550
11	430	1 120	+690
15	340	805	+465
Mean	491	1 145	+654
Significance of drug effect			$p < 0.01$
Placebo			
3	665	615	−50
9	540	575	+35
12	515	600	+85
Mean	573	597	+23
Significance of drug effect			N.S.

week of continuous administration of LB 46, there were only minor changes as compared to the results obtained following acute administration of a single dose. Table II shows that at rest, as well as at every level of work tested, the heart rate tended to be slightly higher after continued use of the drug than following the first single dose. Since the patients were receiving no thyrostatic medication, this increase in the heart rate may have been due to exacerbation of the hyperthyroidism during the experimental period. Adaptation to the drug cannot be excluded, however since no control tests were carried out for the assessment of the basic heart rate without medication at the termination of the experimental period. Thus the experimental procedure does not permit differentiation between these two possibilities. But in any case, if there was adaptation to the drug it was at most very slight, the difference between the results barely reaching statistical significance ($p < 0.05$) at one level of work load.

The most striking effect of LB 46 was the very strong increase in PWC_{170} (Table III). It is further seen from Table IV that this effect persisted essentially unchanged at the end of the experimental period. It should be pointed out, however that the estimation of PWC_{170} was considered appropriate by means of extrapolation (or rarely interpolation) over a narrow interval from the heart rate end point obtained, and that this interval was essentially greater in the tests following medication than in the controls. No substantial change was observed in the actual physical working capacity The change in PWC_{170} induced was therefore mainly due to decrease in the pulse response to physical work.

The effect of LB 46 on the blood pressure was small. Following acute administration there was

Table IV *Comparison of the effects of acute and continuous (1–3 weeks) administration of LB 46 on PWC_{170}*

Pat. no.	PWC_{170} after acute administration–control value (acute dose of LB 46, mg) (kpm/min)		PWC_{170} after continuous administration–control value (duration of medication, d.) (kpm/min)		Difference between the results of continuous and acute administration (kpm/min)
5	680	(5)	680	(7)	0
7	835	(10)	835	(7)	0
8	550	(10)	50	(7)	−500
13	750	(5)	1 050	(7)	+300
14	520	(5)	520	(7)	0
11	690	(10)	510	(11)	−180
15	465	(10)	1010	(11)	+545
4	425	(5)	550	(14)	+125
10	750	(5)	340	(14)	−410
2	710	(10)	560	(21)	−150
Mean	637		610		−27
Significance of difference					N.S.

no statistically significant change in either systolic or diastolic pressure. There was a mean decrease of 9 mmHg (range +10 to −25 mm) in systolic and 4 mm (+10 to −20 mm) in diastolic pressure at rest. Similar changes were measured during work and also at the end of the experimental period.

In two cases in which 10 mg of LB 46 was administered in the acute test, disturbances in cardiac rhythm necessitated the termination of ergometry. One woman, 22, developed bigeminy of 5 min duration at 450 kpm/min, and another woman, 37, had a short attack of supraventricular tachycardia at 300 kpm/min. No complications were observed at rest or during the continuous administration of 5 mg LB 46 every 8 h.

CONCLUSIONS

The present study clearly indicates that LB 46 effectively diminishes tachycardia in hyperthyroidism and that, mainly through this effect, it markedly increases the PWC_{170}. The effect on the heart rate is evident not only during exercise but also at rest in the supine position, in which the sympathetic drive is normally minimal and the effect of LB 46 slight (5). These results are in accordance with those reported for propranolol in hyperthyroidism (2, 10) and for LB 46 in euthyroidism (5). Furthermore, the prolonged administration of LB 46 demonstrates that the drug effect persists during at least one to three weeks of continuous administration. LB 46 may thus be useful in the initial symptomatic treatment of the hyperkinetic circulation in hyperthyroidism before the effect of any thyrostatic measures is evident.

No untoward effects necessitating termination of the medication were encountered during the therapeutic trial. The patients generally reported subjective improvement and no signs of impaired cardiovascular adaptation to work were observed, although they were specifically looked for. The two patients who developed transient cardiac rhythm disturbances during the exercise test after receiving a single dose of 10 mg LB 46 did perfectly well during the subsequent continuous medication with 5 mg every 8 h.

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BETA ADRENERGIC BLOCKADE IN THE TREATMENT OF LEFT-SIDED HYPERTROPHIC OBSTRUCTIVE CARDIOMYOPATHY (HOCM)

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Abstract. The surgical treatment of left-sided HOCM is difficult and disappointing. Favourable results of treatment of this disease with beta-adrenergic blocking drugs have been reported. Therefore the effects on left ventricular haemodynamics of acute and/or long-term treatment with beta-adrenergic blocking drug were studied in eight patients. In some patients only a minor decrease of the left ventriculo-aortic pressure gradient was found. From these results concluded that long-term administration of propranolol in patients with HOCM is of no practical use. *One patient, who had been operated* upon earlier and was also submitted to the experiments, as hardly any improvement took place after the operation, did improve on treatment with propranolol. From these experiments it was concluded that operation is still the treatment of choice for this disease bearing in mind however that HOCM is diffuse myocardiopathy Treatment with propranolol after operation might improve the effect of operative therapy

Pearse (9) found the norepinephrine content and sympathetic innervation in the outflow tract of the left ventricle in patients who died of HOCM (8, 9 13) to be far greater than normal. He called these findings adrenosis In the literature favourable results have been reported from the treatment of HOCM with beta-blocking agents, which might counteract this adrenosis.

Surgical treatment of HOCM of the left ventricle is a difficult procedure, because at operation it is often impossible to see where the out flow tract is narrowed.

Therefore we decided to study in patients with this disease the effects of a beta-blocking agent (propranolol) on heart catheterization in the acute experiment as well as after long-term treatment orally

MATERIAL AND METHODS

Eight patients, five women and three men, participated in this study All eight patients, aged between 22 and 55 had history of HOCM, which as confirmed by physical examination, chest X-ray (Fig. 1), ECG, vectorcardiogram, phonocardiogram (Fig. 2) and heart catheterization. Seven patients had positive family history (early deaths from heart disease re of the patients were sisters). Table I shows the data of these patients. They all had typical vectorcardiogram, simulating myocardial infarction, a typical carotid pulse tracing, showing the bifid carotid pulse with short ejection time, the systolic ejection murmur in the phonocardiogram with *free interval after the first heart sound (Fig 2) and* typical apex cardiogram, showing bifid tracing

On cineangiography (Fig. 3) the outflow tract obstruction could be easily defined in all patients

Right- and left-heart catheterization as performed One catheter (USCI no 7) as introduced via the antecubital vein into the pulmonary artery another (NIH no. 7) via the brachial artery into the left ventricle and third (Ödman catheter) via the femoral artery (using the Seldinger technique) into the ascending aorta.

The following measurements were made: (*a*) heart rate (ECG—Sanborn 560/350/3200); (*b*) left ventricle-aortic pressure gradient (Statham P23–Db and Sanborn 560/350/1100 B); (*c*) cardiac index ml sec m²; (*d*) stroke volume index ml/sec/m²—both measured with Fox dilution curves using the Stewart-Hamilton formula (Photoelectric cell IR Kipp and Sanborn 560/3 0 1500; (*e*) cineangiography using Philips 9-inch image intensifier and Arriflex camera.

RESULTS

In seven patients (patient A did not participate in the acute experiment) these measurements during heart catheterization were made before and 5 10 and 15 min after 5 mg propranolol intravenously (Cineangiography was not repeated after the injection of propranolol.)

The heart rate decreased in four patients (B, C, E, and G) the stroke volume index in two (F and H) (Fig. 4).

Only in three patients (B, G and H) was there



Fig. 1. Chest X-ray of patient B. Enlargement of left ventricle. Normal aorta configuration.

a decrease of the left ventriculo-aortic pressure gradient. The cardiac index decreased in four patients (E, F, G and H). In one patient (D) the cardiac index increased (Fig. 5).

After studying the initial effects of propranolol seven patients were recatheterized, using the same technique as in the acute experiments, after six months treatment with 120–160 mg propranolol orally per day. Six of these patients also participated in the acute experiments (patient F was not recatheterized). The results were compared with those found in the acute experiments before the injection of propranolol.

The heart rate was lower in four patients (A, C, E and G). The cardiac index and stroke volume index were decreased in two patients (E and H), and increased in three (B, D and G) (Fig. 4).

Fig. 6 demonstrates the relation between left ventriculo-aortic pressure gradient and cardiac index before and after long-term treatment with propranolol.

In only three patients (H, D and E) was a lower ventriculo-aortic pressure gradient found than before the long-term treatment.

The complaints of the patients did not change; patients C and G complained of additional fatigue. The findings on physical examination, except for the heart rate in four patients, were not altered. The electro- vector- and phonocardiograms and chest X-rays were the same as before treatment.

DISCUSSION

Goodwin (6) and Cohen and Braunwald (5) reported favourable effects of beta-adrenergic blockade during heart catheterization in patients with HOCM. Scheu et al. (10) found a good effect of long-term treatment with propranolol at recatheterization in one patient. Bowyer et al. (4) reported favourable effects of guanethidine in the acute experiment as well as after long-term treatment in two patients on heart catheterization. He also found improvement on exercise.

Scheu et al. (10), Cohen and Braunwald (5), Sloman (12) and Goodwin (6) described favourable effects of beta-blocking agents on exercise tolerance and complaints in patients with HOCM.

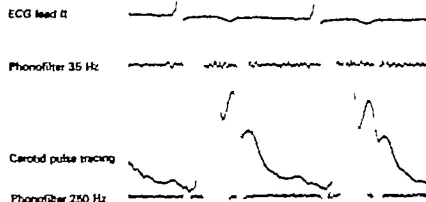


Fig. 2. Carotid pulse tracing and phonocardiogram of patient C.

Table I. *Survey of eight patients with HOCM*

Patient	Age (y.)	Sex	Complaints	Physical signs	ECG	Chest X-ray	LV aortic pressure gradient (mmHg)
A	45	♂	Angina pectoris	Soft 1st heart sound. Systolic murmur grade III with free interval after 1st heart sound. Double ictus impulse	Left strain	Normal	50
B	53	♂	Angina pectoris	Soft heart sounds. Systolic murmur grade III with free interval after 1st heart sound	Left bundle branch block	Enlarged left ventricle. Prominent aorta	80
C	32	♀	Dyspnoea on exertion. Palpitations	Systolic murmur grade I–II with free interval after 1st heart sound	Left strain	Prominent left ventricle	35
D	37	♀	Angina pectoris. Palpitations. Fatigue	Systolic murmur grade IV with free interval after 1st heart sound	Broad Q in I. Ventr. extrasystoles 1st degree A–V block. Left strain	Enlarged left ventricle	120
E	22	♀	Angina pectoris	Loud 1st heart sound. II A loud. Systolic murmur grade IV–V with free interval after 1st heart sound	Left strain	Enlarged ventricle	45
F	45	♂	Angina pectoris	Systolic thrill. Systolic murmur grade IV with free interval after 1st heart sound	Left strain. Left hypertrophy	Enlarged left ventricle	90
G	41	♀	Angina pectoris	Soft 1st heart sound. Systolic murmur grade IV with free interval after 1st heart sound	Left strain. Left hypertrophy	Enlarged left ventricle	120
H	48	♀	Angina pectoris. Palpitations. Fatigue	Loud 1st heart sound. Systolic murmur grade IV with free interval after 1st heart sound. Double ictus impulse	Left strain	Enlarged left ventricle	45



Fig. 3. Cineangiogram of patient G showing the obstruction of the outflow tract of the left ventricle during systole.

In our studies we only saw a decrease of the left ventriculo-aortic pressure gradient in three patients (G, H and B) in the acute experiment. The pressure gradient and cardiac index both increased in patient D after 5 mg propranolol intravenously. This might be explained by relatively greater obstruction of the inflow tract of the left ventricle than of the outflow tract.

After long-term treatment with propranolol in only three patients (H, D and E) a lower left ventriculo-aortic pressure gradient was found. Patient H was operated upon (the dotted column in Fig. 6 shows the measurements before opera-

Congress Announcements

The 16th Congress of Pediatrics with international participation will take place in Prague, Czechoslovakia, 9 to 11 Sept. 1971

President. Prof. Dr F Blažek.

Secretariat: Dr A. Kopecký CSc., Sokolská 31 Prague 2, Czechoslovakia.

Main topics: 1 Growth and development of the child, 2. Nephrology in children, 3 Recent progress in diagnostics and therapy

The Sixth International Congress on Photobiology will be held in Bochum BRD (North-Rhine Westfalia, close to Düsseldorf international air port), August 21 to 25 1972.

President: Prof Dr G O Schenck.

Secretary general. Prof. Dr H. Tronnier Universitäts-Hautklinik, D-7400 Tübingen, West Germany

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ON COAGULATION AND FIBRINOLYSIS IN CONSERVATIVELY TREATED CHRONIC URAEMIA

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Abstract. Factors of the coagulation and fibrinolytic systems have been studied in 50 conservatively treated patients with chronic uraemia of varying origin and serum creatinine levels of 2.1–17.5 mg/100 ml. The patients were grouped according to serum creatinine level, aetiology of renal failure and actual treatment. The investigation showed that decreased platelet adhesiveness, prolonged Ivy bleeding time and bleeding tendency increased content of certain coagulation factors (factor VIII, P & P fibrinogen), decreased fibrinolytic activity and increased content of inhibitors of plasminogen activation were common. The platelet adhesiveness decreased, the bleeding time and the bleeding tendency increased with increasing serum creatinine. Factor VIII, P & P and fibrinogen tended first to rise and then to fall, while the fibrinolytic activity and the content of inhibitors of plasminogen activation did not vary with renal function. No certain correlation was found between the changes observed and the aetiology of uraemia or actual treatment.

Haemorrhagic diathesis is a well known complication of uraemia (1). The introduction of haemodialysis drew attention to haemostasis in uraemia, partly because of the risk of haemorrhagic complications, partly because of the complicating clotting in external shunts. The interest in haemostasis in uraemia has increased still further owing to the possible pathogenetic role played by deposition of fibrin in various renal diseases.

It is known that platelet function may be impaired in uraemia, the impairment being reflected by a prolonged bleeding time (18, 42, 45), by a defective release of platelet factor 3 (2, 15 20, 22, 37 43) and by decreased platelet adhesiveness and aggregation (5 11 14 39 40, 42). Increased fibrinogen and decreased fibrinolytic activity have been observed in animal experiments and in patients with renal disease (9 24 25 26, 44). Discrepancies between data available and recent advances in nephrology prompted us to investigate the coagulation and fibrinolytic systems in uraemia. This paper concerns the behaviour of factors in these systems in conservatively treated chronic uraemia.

MATERIAL

The clinical material consisted of 50 patients with conservatively treated chronic uraemia. The patients ages ranged from 13 to 80 years (mean 48.2). The serum creatinine levels and the aetiology of renal failure are given in Table I and Fig. 1. The diagnosis of pyelonephritis was clinical in 17 cases, based on post mortem findings in 4, and on histological findings in biopsy specimen of the kidney in 1. In the patients with glomerulonephritis the diagnosis was based on the clinical picture in 4, on histological examination in 4, and on post mortem findings in _. The diagnosis of polycystic kidney (11 cases) was based on clinical and roentgen findings. The mixed group (7 cases) included 47-year-old man with insulin-treated diabetes and nephropathy 69-year-old woman with Wegener's granulomatosis (biopsy of nasal mucosa and renal tissue), 52-year-old woman with rheumatoid arthritis and renal amyloidosis (diagnosed post mortem), 37-year-old man with multiple arterial stenoses, including one renal artery and malignant hypertension, 52-year-old man with ankylopoietic spondylarthritis. The aetiology of the nephropathy in the last-mentioned case could not be established, but the patient had been using phenylbutazone for long time. In 2 cases (men, aged 39 and 45) the aetiology of the renal failure was not known with certainty. Renal biopsy had not been performed in these cases.

In the analyses of the findings the patients are grouped according to serum creatinine level, the cause of the renal failure and the actual treatment.

Four patients (serum creatinine 4.2–8.0 mg/100 ml) were treated with acetyl-salicylic acid at the time of the investigation, 7 (serum creatinine 2.1–10.7 mg 100 ml) with corticosteroids and/or ACTH, and 15 (serum creatinine 2.9–13.4 mg/100 ml) had antimicrobial therapy (Ampicillin in 8 cases, V-Penicillin in 1, sulpha and/or Nitrofurantoin in 5, and Nalidixin in 1). One patient who had

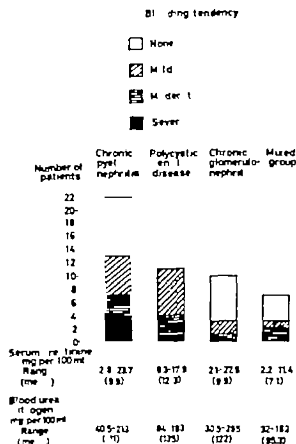


Fig. 1. Bleeding tendency in the different aetiological groups.

not only non-specific pyelonephritis, but also tuberculosis of the urinary tract was treated with Streptomycin + Natrium PAS + Ampicillin. In that case the values for clotting time, factor V and P & P were not included in the analysis of the findings, and in one case in which the patient was receiving treatment with dicoumarol the clotting time and P & P were not included. In patients with severe uraemia the intake of protein was limited to 50 g per day and patients with tendency to oedema or hyperkalaemia were given a low-sodium and low-potassium diet, respectively.

METHODS

Collection of blood. The blood was collected by the silicone technique and citrated plasma and serum were prepared as described previously (29, 35).

Coagulation studies. The following determinations were made: bleeding time, platelet count, platelet adhesiveness in whole blood, clotting time in glass and plastic tubes, prothrombin + factor VII + factor X (Owren P & P test), factor V, AHF (factor VIII), fibrinogen and thrombin time.

Platelet adhesiveness was measured by the method of Hellem (13), as modified by Cronberg et al. (6), and Duke and Ivy bleeding times as described by Nilsson et al. (30). The thrombin time was determined in the way described by Nilléhn and Nilsson (28).

The fibrinogen was determined by the technique described by Nilsson and Olow (31). Only fibrinogen values for blood collected with E-ACA are given.

Other determinations were performed by methods described by Nilsson et al. (29).

Fibrinolytic studies. The following determinations were

Table I. *Frequencies and means of abnormal findings*

	Frequency of abnormal findings ( )			
	> Normal	< Normal	Mean ± S.D	Normal range
Serum creatinine (mg/100 ml)			10.0 ± 5.7	0.6–1.2
BUN (mg/100 ml)			116 ± 56	10–20
Platelet count (10³/µl)		28	232 ± 112	200–400
Bleeding time (min)				
Duke	21		4.6 ± 4.4	1 5
Ivy	59		16.6 ± 8.0	6–1.
Platelet adhesiveness ( )		28	26 ± 9	20–52
Clotting time (min)				
Glass	15	2	11.9 ± 2.3	8–14
Plastic	60		27.5 ± 6.6	15–25
Factor VIII (%)	74	0	252 ± 124	60–160
P & P (%)	56	0	130 ± 29	80–120
Factor V (%)	22	8	106 ± 22	80–120
Fibrinogen (g/100 ml)	90	0	0.57 ± 0.18	0.20–0.40
Plasminogen (%)	12	0	112 ± 31	60–140
Urokinase inhibitors (%)	78	0	203 ± 86	60–140
α_2-macroglobulin (%)	20	26	101 ± 37	80–120
Thrombin time	32			

urokinase fibrinolytic activity of plasma and resuspended euglobulin precipitate on unheated fibrin plates, inhibitors of urokinase activation of plasminogen (urokinase inhibitors), α_2-macroglobulin and fibrinolytic split products. The methods described earlier (27, 32, 33, 35) were used. α_2-macroglobulin was determined by the method of Ganrot (12).

Other laboratory investigations were performed by routine methods used at the Central Laboratory of Clinical Chemistry at the Lund University Hospital.

In the statistical analysis of the findings comparison was made between the mean values in the various aetiological groups and at different serum creatinine levels, besides which the coefficients were calculated for correlations between factors of the coagulation and fibrinolytic system, on the one hand, and age, blood urea nitrogen (BUN), serum creatinine, serum uric acid, Hb, haematocrit, serum electrolytes, serum proteins, serum iron TIBC, cholesterol and triglycerides, on the other as well as between the factors of the coagulation and fibrinolytic systems themselves. Measurement of Ivy bleeding time was limited to 30 min, which may have affected the results of the statistical analysis.

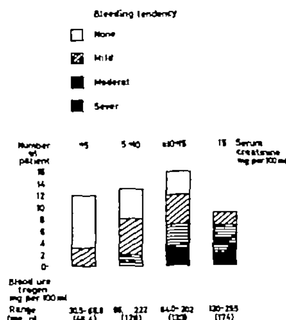


Fig. 2. Bleeding tendency at different serum creatinine levels.

RESULTS

All correlations with $p < 0.05$ are given in Table IV. Correlations of special interest are given in the text and some also diagrammatically.

1. *Bleeding tendency* (Figs. 1 and 2, Table III). The haemorrhage was generally confined to the mucosae or skin, and one patient had a subdural haematoma. The bleeding tendency was said to be mild when there was recurrent bleeding from one source; moderate when the bleeding derived from two sources and severe when there were several sources or when the blood loss was clinically important, e.g. required blood transfusion. A bleeding tendency was noted in 30 (60%) cases. It was severe in 5 (10%), moderate in 9 (18%) and mild in 16 (32%). The last-mentioned group included 5 patients with polycystic kidneys and recurrent haematuria without other bleeding symptoms.

Table II. *Means with S.D. of coagulation and fibrinolytic parameters and number of cases with prolonged thrombin time at different creatinine levels*

	Serum creatinine (mg/100 ml)			
	<5	≥5 <10	≥10 <15	>15
No. examined	12	13	16	9
BUN (mg/100 ml)	45.4±10.4	126±44	130±34	174±43
Platelet count (10³/µl)	234±75	326±152	230±76	179±69
Bleeding time (min)				
Duke	3.1±0.6	3.3±2.1	6.1±6.8	5.2±3.0
Ivy	12.6±5.0	16.4±8.0	17.3±7.7	20.5±10.5
Platelet adhesiveness (%)	34±5	25±8	23±8	24±13
Factor VIII (%)	208±109	284±142	268±115	235±129
P&P (%)	131±21	139±26	129±35	117±28
Factor V ( )	114±23	111±27	102±18	95±16
Fibrinogen (g/100 ml)	0.50±0.13	0.66±0.19	0.57±0.20	0.53±0.14
Plasminogen ( )	111±22	112±35	123±37	95±17
Urokinase inhibitors ( )	191±66	224±127	194±82	204±38
α_2-macroglobulin (%)	99±12	97±32	113±49	81±39
Thrombin time prolonged in	4 of 12	4 of 13	4 of 16	4 of 9

Table III. *Frequency of some coagulation defects in 30 patients with and 20 patients without bleeding symptoms. The difference in Ivy bleeding time was almost significant ($p < 0.05$)*

	Prolonged Duke bleeding time	Prolonged Ivy bleeding time	Decreased platelet adhesiveness	Increased factor VIII	Increased P & P	Increased fibrinogen	Increased urokinase inhibitors
Patients with bleeding symptoms	7/30	1/29	9/30	23/30	19/30	27/30	22/30
Patients without bleeding symptoms	3/18	6/17	5/20	12/20	8/18	18/20	17/20

Twenty-one of 29 cases with a clinical bleeding tendency had a prolonged Ivy bleeding time. The distribution of the patients with a tendency to bleeding among the aetiological groups is given in Fig. 1 The severity of the bleedings increased with the serum creatinine level (Fig. 2) and the BUN was numerically higher among patients with bleeding tendency than among those without (mean $\pm$S.D = 128 $\pm$48 and 100$\pm$63 mg/100 ml, respectively $0.05 < p < 0.1$). Table III gives a comparison of the coagulation defects between patients with and without bleeding symptoms. The difference for Ivy bleeding time was nearly significant ($p < 0.05$), while the other variables showed only small numerical differences.

2. *Duke bleeding time* (Tables I II, III and IV) The bleeding time determined by Duke's method was prolonged in one fifth of the cases. All the cases with a prolonged Duke time had also a prolonged Ivy time. Patients with serum creatinine $\geq$10 mg/100 ml had an almost significantly higher mean value than patients with a lower creatinine value ($p < 0.05$). The Duke bleeding time increased significantly with decreasing platelet adhesiveness ($p < 0.01$) and increasing α_1-globulins ($p < 0.001$).

3 *Ivy bleeding time* (Tables I, II, III and IV). The bleeding time according to Ivy's method was prolonged in 27 of 46 cases. It increased significantly with the serum creatinine ($p < 0.01$) and its positive correlation with the BUN and with α_1-globulins was nearly significant ($p < 0.05$). A negative correlation ($p < 0.001$) was found, with platelet adhesiveness (Fig. 3) and with Hb and haematocrit, respectively ($p < 0.01$).

4. *Platelet count* (Tables I and II). Twelve of 47 patients had more than 100 000 and less than 200 000 platelets per μl and one had 70 000. The remaining patients had more than 200 000. The patient with 70 000 platelets per μl was a 33-year-old man with chronic glomerulonephritis and rapidly progressive uraemia (serum creatinine 27.5 BUN 255 mg/100 ml, platelet adhesiveness 0% Ivy bleeding time >30 min).

The platelet count first increased but then decreased with increasing serum creatinine. The decrease was significant ($p < 0.01$).

5 *Platelet adhesiveness* (Tables I, II, III and IV). In 14 patients platelet adhesiveness was decreased. It decreased with increasing BUN (Fig. 4 $p < 0.001$) and serum creatinine ($p < 0.01$) A positive correlation was found between platelet adhesiveness and Hb ($p < 0.001$) and haematocrit ($p < 0.01$), respectively Eight of 27 cases with prolonged Ivy bleeding time had decreased platelet adhesiveness, compared with 5 of 10 cases with a prolonged Duke time.

6. *Clotting time in glass and plastic tubes* (Tables I and IV). Seven of 47 patients had a prolonged clotting time in glass, compared with 29 of 48 when plastic tube was used. In one case the value was slightly shorter than normal.

7 *Factor VIII* (Tables I, II, III and IV). In 37 of 50 cases factor VIII was increased and tended to increase, though not significantly with the serum creatinine to 10 mg/100 ml, and afterwards to fall. It varied significantly ($p < 0.01$) with α_1-globulins (Fig. 5) and showed a nearly significant positive correlation ($p < 0.05$) with fibrinogen.

8. *Owren's P & P* (Tables I, II, III and IV). P & P was increased in 27 of 48 cases and normal in the others. When the serum creatinine was $\geq$15 mg/100 ml, the mean was numerically lower than when the serum creatinine was <15 mg/100 ml ($0.1 < p < 0.2$).

Table IV *Coefficients of correlation (p < 0.05). Coefficient with p < 0.001 are given in italics*

	Clotting time, plain	Clotting time, plastic	Duke bleeding time	Ivy bleeding time	Platelet adhesiveness	Factor VIII	P&P	Factor V	Fibrinogen	Plasminogen	Urokinase inhibitors	α_2-macroglobulin
Age					0.29 $p<0.05$							*−0.59 p < 0.001*
Serum creatinine				0.37 p 0.01	−0.42 $p<0.01$							
BUN			0.30 $p<0.05$	0.30 p 0.05	*−0.51 p < 0.001*						0.29 $p<0.05$	
Calcium/s		0.29 p 0.05	*0.49 p 0.001*						−0.33 $p<0.05$			
Phosphorus/s			0.35 $p<0.05$									
[Po]tassium/s	0.37 p 0.05											
[So]dium/s			−0.31 p 0.05									
[C]hlorides/s			−0.46 p 0.01									
Magnesium/s									−0.39 $p<0.05$			
Albumin									−0.34 $p<0.05$			
$_1$-globulins			*0.50 p 0.001*	0.34 p 0.05		0.38 p 0.01			0.36 $p<0.05$			
β_1-globulins		0.30 $p<0.05$	0.32 p 0.05				0.33 p 0.05					
Triglycerides			0.47 $p<0.05$						0.40 $p<0.05$			
Haemoglobi[n]			−0.28 p 0.05	−0.43 $p<0.01$	*0.51 p 0.001*							
Haematocrit			0.31 p 0.05	−0.44 p 0.01	0.47 p 0.01							
Clotting time plastic	*0.57 p < 0.001*											
Duke bleeding time				*0.71 p < 0.001*	−0.41 p 0.01			−0.30 $p<0.05$	0.44 p 0.01	0.33 p 0.05		
[Pla]telet adhesiveness				*0.54 p 0.001*						*−0.45 p < 0.001*		
[F]actor VIII									0.32 $p<0.05$			

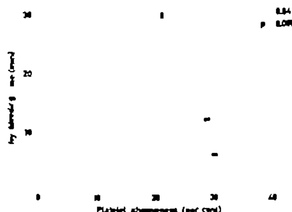


Fig 3 Negative correlation between Ivy bleeding time and platelet adhesiveness.

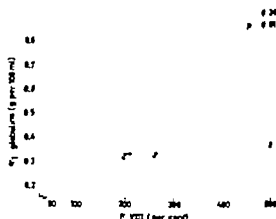


Fig 5 Positive correlation between factor VIII and α_1-globulins.

9 *Factor V* (Tables I, II and IV) Factor V was normal in 34 patients, increased in 11 and decreased in 4 Like P & P factor V tended to decrease at high serum creatinine level with a lowest mean at a creatinine value of ≥ 15 mg/ 100 ml.

10 *Fibrinogen* (Tables I II III and IV). Fibrinogen was increased in 45 cases and normal in the remaining 5 The values increased almost significantly with the serum creatinine to 10 mg/ 100 ml ($p < 0.025$) and afterwards fell, though not significantly with increasing creatinine. The positive correlations with α_1-globulins (Fig. 6) and factor VIII and the negative with albumin were nearly significant ($p < 0.05$). The relation to α_2-globulins is given in Fig. 7

11 *Fibrinolytic activity* (Fig. 8) The fibrinolytic activity of euglobulin precipitate (and of plasma, not included in figure) was lower in patients with uraemia than in normals. No correlation was found with the serum creatinine or the aetiology of uraemia.

12. *Plasminogen* (Tables I, II and IV). The plasminogen level was normal in 44 patients and raised in the remaining 6 The mean was lowest in the group with serum creatinine of ≥ 15 mg/100 ml. The difference between this group and the group with serum creatinine $\geq 10 < 15$ mg/100 ml was almost significant ($p < 0.05$)

13 *Urokinase inhibitors* (Tables I, II III and IV) The content of inhibitors of urokinase activa-

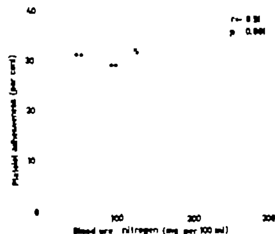


Fig 4 Negative correlation between blood urea nitrogen and platelet adhesiveness. If one case with BUN of 255 mg/100 ml and platelet adhesiveness of 0% is excluded, the coefficient of correlation will be 0 43 with $p < 0.005$

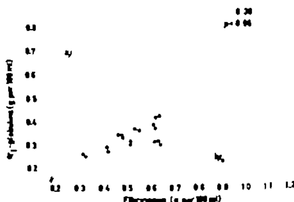


Fig 6. Almost significantly positive correlation between fibrinogen and α_1-globulins for the entire series. Two cases (and *b*) differ from the others. If one of these (*a*) is excluded, the correlation coefficient will be 0.46 with $p < 0.005$ and, if both are excluded, 0 61 with $p < 0.001$.

tion of plasminogen was increased in 39 and normal in the remaining 11. No significant variation with serum creatinine was found.

14 *α_2-macroglobulin* (Tables I, II and IV). The α_2-macroglobulin did not vary in any particular way in the material as a whole. It is true that the values for 16 of 35 patients lay outside the normal range, but the deviations were small except in three cases, two with high values and one with a low value. The high values must be regarded as relatively normal because the patients were below 20 years of age. The mean tended to be lower among patients with high serum creatinine.

15 *Thrombin time* (Tables I and II). The thrombin time was prolonged in 16 patients. There was no clear correlation with the serum creatinine. A prolonged thrombin time was found in 7 of 12 patients with fibrinolytic split products compared with 6 of 28 without. The difference was almost significant ($p < 0.025$).

Prolonged Ivy bleeding time tended possibly to be more common in chronic pyelonephritis, occurring in 14 of 19 cases compared with 5 of 10 cases with chronic glomerulonephritis and 6 of 11 cases with polycystic kidney. There was, however, no significant correlation between the disorders in the coagulation and fibrinolytic systems and type of renal disease. Nor did the treatment the patients were receiving at the time of the investigation appear to have any effect.

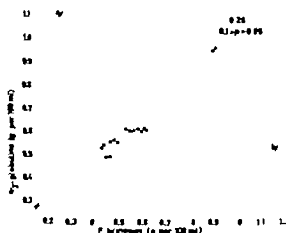


Fig. 7. No significant correlation between fibrinogen and α-globulins for the total material. If one (a) of the two deviating cases is excluded, there will be a significant positive correlation with a coefficient of 0.53 and $p < 0.001$.

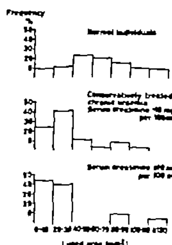


Fig. 8. Fibrinolytic activity measured as lysis of resuspended euglobulin precipitate on unheated fibrin plates in normal individuals and in conservatively treated chronic uraemia. The uraemic patients are divided into two groups with serum creatinine above and below 10 mg/100 ml.

DISCUSSION

Decreased platelet adhesiveness, prolonged Ivy bleeding time and bleeding tendency, increased amount of certain coagulation factors (factor VIII, P & P, fibrinogen), decreased fibrinolytic activity and increased content of inhibitors of plasminogen activation were found to be common in conservatively treated chronic uraemia.

The clinical tendency to bleeding was found to be correlated with the degree of renal insufficiency but there was no certain correlation with the aetiology of uraemia.

The investigation confirmed that Ivy's method for determination of bleeding time is more sensitive than Duke's technique (30). The platelet count was largely normal, and the bleeding time, according to Ivy and Duke, was negatively correlated with platelet adhesiveness, which suggests that the prolongation of the bleeding time was due to decreased adhesion and aggregation of the platelets. No correlation was found between the serum calcium and platelet adhesiveness. On the other hand, platelet adhesiveness was dependent on renal function since a negative correlation was found between platelet adhesiveness and serum creatinine and BUN respectively. Judging from previous investigations, it is probable that it is not the urea per se, but some simultaneously re-

tained substance, possibly guanidinosuccinic acid, that influences platelet function (4 15 41) A therapeutic consequence of the reduced platelet adhesiveness is that dextran 70 is contraindicated in the presence of haemorrhage in patients with uraemia (5) and that caution should be exercised in the use of certain types of analgetics, particularly preparations containing acetyl-salicylic acid (34)

As regards the behaviour of coagulation factors, opinions differ except for fibrinogen. Thus certain authors have found an increased factor VIII activity in some cases (7 10, 16 36), while others have found only normal values (22) or a reduced value in some cases (38). For the prothrombin group reduced values have most often been found, but in varying frequency (3 8 38). Kuhlbäck has, however reported increased values for prothrombin (19).

The increase of factor VIII and fibrinogen demonstrated in the present investigation may be explained by an increased new formation associated with a reactive process. The correlation with α_1-globulins may favour this assumption. If it is due to a reactive process, this may also be extrarenal since we have found increased factor VIII and fibrinogen also in patients who had undergone bilateral nephrectomy (21). The decrease at high serum creatinine level may be due to a decreasing new formation in severe renal insufficiency (23). The concentration of serum albumin and total protein was not decreased, which may argue against haemodilution as an explanation.

Our investigation showed, like previous studies, that the fibrinolytic activity is low in chronic uraemia. It has previously been stated that the decrease of the fibrinolytic activity in impaired renal function is due to decreased production of urokinase or other fibrinolytic activators in the damaged kidney (26). Isacson and Nilsson (17) however found that patients who had been subjected to bilateral nephrectomy more than one year previously could develop fibrinolytic activity during venous occlusion and had a histochemically normal content of plasminogen activators in the walls of the veins. These investigations argue against any essential role of the kidneys in the production of plasminogen activators and against the decrease of fibrinolytic activity in uraemia being due to decreased activator content. The present investigation also showed that the low fibrinolytic activity does not depend on an increased content of α_2-macroglobulin. On the other hand it appears very probable that it has something to do with the marked increase in the inhibitors of plasminogen activation observed by us. This increase, like the fibrinolytic activity was not certainly correlated with renal function, and may be a link in a reactive process, like the increase of factor VIII and fibrinogen.

The low fibrinolytic activity may have pathophysiological consequences. It is very probable that it plays a role in the underlying mechanism of the deposition of fibrin in the kidneys in glomerulonephritis, and possibly also in other renal diseases, as well as in the formation of extrarenal deposition of fibrin in uraemia.

ACKNOWLEDGEMENTS

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ON COAGULATION AND FIBRINOLYSIS IN ACUTE RENAL INSUFFICIENCY

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Abstract. Factors of the coagulation and fibrinolytic systems have been studied in 18 patients with acute renal insufficiency 13 with acute tubular necrosis and 5 with glomerulonephritis. In six cases there was more or less pronounced thrombocytopenia, but in none of the patients were there certain signs of disseminated intravascular coagulation. The general picture of the coagulation and fibrinolytic systems in acute uraemia found in the present investigation was characterized by prolonged Ivy bleeding time, increased content of factor VIII and fibrinogen, low fibrinolytic activity and increased value for inhibitors of plasminogen activation, largely the same changes as those found in chronic uraemia.

Knowledge *of disorders of haemostasis is desirable* in the treatment of acute renal insufficiency which may require haemodialysis and reoperation in some cases and corticosteroid therapy or anticoagulants in others. In most investigations of coagulation disturbances no distinction has been made between acute and chronic uraemia. According to available experience, acute uraemia is associated with a clinical tendency to bleeding, thrombocytopenia, impaired platelet-factor 3-function, prolonged bleeding time, increased fibrinogen, decreased values of the factors in the prothrombin group and decreased fibrinolytic activity (4 7 14 17 34 35 38, 39). But the results obtained in earlier studies differ in various respects.

We studied the coagulation and fibrinolytic systems in patients with acute uraemia and compared the results with those obtained in a separate investigation of conservatively treated chronic uraemia (18).

MATERIAL

The material consisted of 18 patients, 9 women and 9 men. The age distribution, serum creatinine levels and blood urea nitrogen (BUN) are given in Table I. The cause of the uraemia was acute tubular necrosis in 13 cases and glomerulonephritis in 5. The acute tubular necrosis occurred as complication of gastric resection 1 extirpation of pancreatic insuloma 1 resection of intestinal fistula 1 vesicolithotomy and resection of sclerosed neck of the urinary bladder 1 and cholecystectomy 1 severe gastroenteritis 3 enterocolitis 1 appendicitis 1, septicaemia caused by Staph. aureus E. coli? 1 eclampsia 1 and myocardial infarction 1 case. Out of 5 cases with glomerulonephritis the condition was acute in 3 an acute exacerbation of subchronic glomerulonephritis in 1 and acute-subacute in 1 case

Seven patients (nos. 1 8, 9 10, 13 15 16) were given 1 16 dialyses with disposable artificial kidney of Alwall type (2), blood pump and Teflon-Silastic shunt (Quinton-Scribner). Two of these patients (nos. 13, 15) had been treated 36 hours and 10 hours, respectively before the first examination of the coagulation system. One patient (no. 14) had been treated for 14 hours with peritoneal dialysis, receiving about 500 IU heparin and 25 000 KIU Trasylol per hour via the dialysis fluid. The values found for the clotting time, fibrinolytic activity fibrinolytic split products, plasminogen, inhibitors of plasminogen activation and thrombin time in that case were not included in the compilation of the results

Three patients (nos. 1 5 14) were being treated with corticosteroids at the time of the first analysis of the coagulation system, and in one of these patients (no 1), in whom the investigation was repeated later heparin was given for nine days after the first examination. This case is also described in separate paper (20). Fourteen patients were receiving broad spectrum antibiotics, mostly Ampicillin, and of the other patients one (no. 1) was receiving V-penicillin and one (no. 4) sulphia. Two patients (nos. 2 and 17) were not receiving any antimicrobial therapy at the time of the study of the coagulation system.

METHODS

Collection of blood The blood was collected by the silicone technique, and citrated plasma and serum were prepared as described previously (27 33).

Coagulation studies. The following determinations were made bleeding time, platelet count, platelet adhesive-

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We studied the coagulation and fibrinolytic systems in patients with acute uraemia and compared the results with those obtained in a separate investigation of conservatively treated *chronic* uraemia (18).

MATERIAL

The material consisted of 18 patients, 9 women and 9 men. The age distribution, serum creatinine levels and blood urea nitrogen (BUN) are given in Table I. The cause of the uraemia was acute tubular necrosis in 13 cases and glomerulonephritis in 5. The acute tubular necrosis occurred as a complication of gastric resection 1 extirpation of pancreatic adenoma 1 resection of intestinal fistula 1 ureterolithotomy and resection of sclerosed neck of the urinary bladder 1, and cholecystectomy 1, severe gastroenteritis 3 enterocolitis 1 appendicitis 1 septicaemia caused by Staph. aureus E. coli? 1 eclampsia 1 and myocardial infarction 1 case. Out of 5 cases with glomerulonephritis the condition was acute in 3, an acute exacerbation of subchronic glomerulonephritis in 1 and acute–subacute in 1 case.

Seven patients (nos. 1, 8 9 10, 13, 15, 16) were given 1–16 dialyses with disposable artificial kidney of Alwall type (2), blood pump and Teflon-Silastic shunt (Quinton-Scribner). Two of these patients (nos. 13 15) had been treated 36 hours and 10 hours, respectively before the first examination of the coagulation system. One patient (no. 14) had been treated for 14 hours with peritoneal dialysis, receiving about 500 IU heparin and 25 000 KIU Trasylol per hour via the dialysis fluid. The values found for the clotting time, fibrinolytic activity fibrinolytic split products, plasminogen, inhibitors of plasminogen activation and thrombin time in that case were not included in the compilation of the results.

Three patients (nos. 1 5 14) were being treated with corticosteroids at the time of the first analysis of the coagulation system, and in one of these patients (no. 1), in whom the investigation was repeated later heparin was given for some days after the first examination. This case is also described in a separate paper (20). Fourteen patients were receiving broad spectrum antibiotics, mostly Ampicillin, and of the other patients one (no. 1) was receiving V-penicillin and one (no. 4) sulpha. Two patients (nos. 2 and 17) were not receiving any antimicrobial therapy at the time of the study of the coagulation system.

METHODS

Collection of blood. The blood was collected silicone technique, and citrated plasma and ... prepared as described previously (27 33).

Coagulation studies. The following made: bleeding time, platelet cou

Table I *Laboratory findings in 18 cases of acute renal insufficiency*

Case no.	Sex	Age (y.)	Serum creatinine (mg/100 ml)	BUN (mg/100 ml)	Hb (g/100 ml)	Platelet count ($10^9/\mu l$)	Bleeding time (min) Duke	Ivy	Platelet adhesiveness (%)	Clotting time (min) Glass	Plastic	Factor VIII (%)	P & P (%)	Factor V (%)	Fibrinogen (g/100ml)
1	♀	13	12.5	162	11.0	190	1.5	30	31	11	23	—	100	85	0.39
2	♀	68	16.6	165	6.2	192	5.0	30	32	12	25	315	90	65	0.49
3	♀	15	8.0	97	9.3	44	20 0	30	10	18	33	170	68	175	0.27
4	♀	25	11.6	85	10.9	72	3.0	15	32	15	—	108	200	150	0.60
5	♀	46	23.4	209	11 7	420	4 4	16	25	12	18	415	81	115	0.67
6	♀	52	3.1	88	7.5	23	10.0	>25	4	15	35	410	66	98	0.99
7	♀	63	9 6	164	10.4	93	3.0	30	36	13	32	460	100	147	0.79
8	♀	63	12.2	153	9 6	168	4.0	30	39	12	24	200	114	125	0 42
9	♀	63	14.2	150	13.2	400	3.7	9	35	12	46	170	160	93	0.45
10	♂	35	9.3	105	8 9	350	4.0	14	46	12	21	188	85	100	0.72
11	♂	34	15.8	114	10.7	420	4.1	13	27	11	24	225	93	94	0.99
12	♂	44	23.0	190	10.0	140	10.0	30	15	12	30	163	103	10	0.69
13	♂	43	11.3	132	—	80	3.8	9	35	9	26	208	112	48	0.36
14	♂	48	12.5	189	7 1	130	6.0	30	12	—	—	350	52	20	0 23
15	♂	49	15 9	225	10.5	276	3.3	18	30	14	30	200	106	72	0 83
16	♂	60	14.6	161	11.6	70	2.5	30	52	12	15	210	90	78	0.61
17	♂	68	8.3	138	10.8	222	2.5	13	45	12	23	475	115	118	0.99
18	♂	71	5 4	72	10.5	240	3.0	13	38	12	30	124	116	115	0 80

↑ = increased, N = normal

ness in whole blood, clotting time in glass and plastic tubes, prothrombin + factor VII + factor X (Owren's P & P test), factor V AHF (factor VIII), fibrinogen and thrombin time. Unless otherwise stated, the methods described previously were used for the determinations (28). *The platelet adhesiveness* was measured by the method of Hellem (9), as modified by Cronberg et al. (3). *The thrombin time* was determined as described by Nilehn and Nilsson (26), *the fibrinogen* as described by Nilsson and Olow (30). Only fibrinogen values for blood collected with E-ACA are given. *The Duke and Ivy bleeding times* were measured as described by Nilsson et al. (29).

Fibrinolytic studies. The following determinations were made: fibrinolytic activity of plasma and resuspended euglobulin precipitate on unheated fibrin plates, plasminogen, inhibitors of plasminogen activation by urokinase (urokinase inhibitors), α_2-macroglobulin and fibrinolytic split products. The methods described earlier (25 31 32, 33) were used. α_2-macroglobulin was determined by the method of Ganrot (6).

In the statistical treatment the data of the coagulation and fibrinolytic systems were compared with one another as well as with the patients' ages, serum creatinine, blood urea nitrogen (BUN), serum uric acid, serum electrolytes, serum proteins, total serum bilirubin, SGOT SGPT and alkaline phosphatase. Reading of the Ivy bleeding time was limited to 30 min, and though about 40% of the patients had an Ivy bleeding time of 30 min or more, the usual correlation coefficient was calculated. Only correlations with $p < 0.05$ and/or of special interest are given in the text. The results obtained in the present investigation were compared with those found in a study of 50 cases of conservatively treated chronic uraemia of varying aetiology and with serum creatinine levels of 2.1 27.5 mg/100 ml (18). This investigation of chronic uraemia showed that decreased platelet adhesiveness, prolonged Ivy bleeding time and bleeding tendency increased content of factor VIII, P & P and fibrinogen, decreased fibrinolytic activity and increased content of inhibitors of plasminogen activation were common.

RESULTS

1 *Bleeding tendency* Seven patients had bleeding symptoms. In two (nos. 2, 13) the bleeding was confined to the gastro-intestinal tract. One of these patients (no. 2) was found to have a hiatus hernia. In one case (no. 15) there was diffuse haemorrhage from the surgical wound when the Teflon-Silastic shunt was inserted, but otherwise no clinical tendency to bleeding. Four of the patients (nos. 3 6 12, 14) had severe haemorrhagic diathesis (diffuse mucosal bleedings, cu-

Fibrinogen (*)	Urokinase inhibitors (%)	α_2-macroglobulin (%)	Thrombin time	Fibrinolytic split products (mg/100 ml)	Total serum bilirubin (mg/100 ml)	SGOT (U)	SGPT (U)	Serum alkaline phosphatase (U)	Diagnosis
86	296	172	I	3.0	—	—	—	—	Acute glomerulonephritis
136	266	73	I	2.3	—	—	—	—	Subacute glomerulonephritis
44	335	109	N	2.5	—	—	—	—	Acute tubular necrosis
74	206	74	I	7.5	0.5	70	130	1	Acute tubular necrosis
104	188	—	N	1.0	—	10	11	—	Acute tubular necrosis
67	310	48	N	7.2	1.2*	63	88	5	Acute tubular necro
117	311	108	N	0	0.8	10	27	1	Acute ubular ecr
87	270	70	N	2.0	0.4	9	18	2	Acute ubular necros
96	184	—	N	—	0.4	5	28	8	Acute tubular necrosis
180	148	—	I	6.0	0.4	15	12	—	Acute glomerulonephritis—diabetes mellitus
127	330	—	I	—	0.3	10	15	5	Acute glomerulonephritis
108	147	—	N	—	—	16	19	—	Subchronic glomerulonephritis with acute exacerbation
[illegible]	305	—	I	—	0.9	44	27	3	Acute tubular necros
—	—	—	—	—	4.5*	75	76	1	Acute tubular necrosis
123	130	—	—	—	2.5*	12	120	6	Acute tubular necrosis
71	251	80	I	8.4	0.6	45	60	14	Acute tubular necrosis
95	255	108	I	traces	—	14	38	7	Acute tubular necrosis
101	250	87	—	0	0.6	44	120	8	Acute tubular necrosis

* Positive direct reaction.

taneous bleedings, post-puncture bleedings) three of these died in the acute stage (nos. 6, 12, 14). Three of these four patients with severe bleeding tendency had an Ivy bleeding time of 30 min or more, and one of them more than 25 min. All of them had reduced platelet adhesiveness, and in two there was also marked thrombocytopenia.

2. *Shunt clotting.* In two of the seven patients (nos. 9, 13) with an external arteriovenous shunt, clotting often occurred in the shunt. There was no local infection and the blood flow was satisfactory. These two patients, who were dialysed 7 and 16 times, respectively, were the only ones with a normal Ivy bleeding time (Table I). One of them (no. 13) had a mild thrombocytopenia and a decreased content of factor V, but otherwise their pattern of coagulation and fibrinolytic systems was largely the same as in the rest of the entire series.

3. *Duke bleeding time* (Tables I and II). A prolonged Duke bleeding time was found in 4 of the 18 cases. In these patients the Ivy bleeding time was also prolonged and platelet adhesiveness decreased. In two there was marked thrombocytopenia. The Duke bleeding time was negatively correlated with platelet adhesiveness ($p < 0.001$). No difference was found between acute and chronic uraemia.

4. *Ivy bleeding time* (Tables I and II). The Ivy bleeding time was prolonged in all cases except two (nos. 9, 13). In these two patients platelet adhesiveness was normal, but there was mild thrombocytopenia in one of them. Four of the 16 patients with prolonged Ivy bleeding time had decreased platelet adhesiveness. Four of the remaining 12 patients with prolonged Ivy bleeding time and normal platelet adhesiveness had less than 100 000 platelets per μl (70 000–93 000), the others more than 100 000.

The mean Ivy bleeding time was almost significantly longer in acute than in chronic uraemia ($p < 0.05$). There was a nearly significant negative correlation of Ivy bleeding time with Hb and platelet count, respectively ($p < 0.05$). Neither the

Table II. *The occurrence of abnormal findings and means of laboratory values in the present material compared with a previously investigated material of conservatively treated chronic uraemia*

Values with significant or nearly significant differences are given in italics

	Acute uraemia			Conservatively treated chronic uraemia			
	Abnormal findings in			Abnormal findings in			
	> Normal	< Normal	Mean ± S.D	> Normal	< Normal	Mean ± S D	Normal range
Serum creatinine			12.6 ± 5.3			10.0 ± 5 7	0.6–1.2 mg/100 ml
BUN			144 ± 44			116 ± 56	10–20 mg/100 ml
Platelet count		11 of 18	196 ± 131		13 of 47	252 ± 112	200–400 10³/µl
Bleeding time							
Duke	4 of 18		5.2 ± 4.3	10 of 48		4.6 ± 4 4	1 5 min
Ivy[a]	16 of 18		*21 4 ± 8.6*	27 of 46		*16.6 ± 8 0*	6–12 min
Platelet adhesiveness		4 of 18	30 ± 13		14 of 50	26 ± 9	20–52 %
Clotting time							
Glass	3 of 17	0	12.6 ± 1.9	7 of 47	1 of 47	11.9 ± [illegible]3	8–14 min
Plastic	8 [illegible] f 16	0	26.9 ± 7.3	29 of 48	1 of 47	27.5 ± 6.6	15–25 min
Factor VIII	15 of 17	0	258 ± 120	37 of 50	0	252 ± 1 4	60–160 %
P & P[b]	2 of 18	3 of 18	*103 ± 34*	27 of 48	0	*130 ± 29*	80–120 %
Factor V	4 of 18	6 of 18	95 ± 43	11 of 49	4 of 49	106 ± 22	80–120 %
Fibrinogen	14 of 18	0	0.61 ± 0.23	45 of 50	0	0.57 ± 0.18	0.20–0.40 g/100 ml
Plasminogen[a]	0	1 of 17	*93 ± 25*	6 of 50	0	*112 ± 31*	60–140 %
Urokinase inhibitors[a]	16 of 17	0	*258 ± 93*	39 of 50	0	*203 ± 86*	60–140 %
α_2-macroglobulin	1 of 10	4 of 10	93 ± 34	7 of 35	9 of 35	101 ± 37	80–120 %
Thrombin time	8 of 15			16 of 50			

[a] $p < 0.05$ [b] $p < 0.005$.

Duke nor the Ivy bleeding time was correlated with the serum creatinine or BUN.

5 *Platelet count* (Tables I and II). Five patients had less than 200 000 but more than 100 000 platelets per µl and six had less than 100 000. The mean tended to be lower in acute uraemia than in chronic ($0.05 < p < 0.1$) All the patients with a platelet count of less than 100 000 had acute tubular necrosis. Five of them had a prolonged Ivy bleeding time. In four of them one of the coagulation factors was decreased and in one also the plasminogen. In none of these patients with a platelet count of less than 100 000 was there, however a simultaneous decrease of factor VIII, P & P factor V and fibrinogen.

6. *Platelet adhesiveness* (Tables I and II). Platelet adhesiveness was decreased in 4 of the 18 patients, compared with 14 of 50 in chronic uraemia. There was no difference, however in the number of adhesive platelets (mean values ± S.D 63 ± 47 and 64 ± 36 × 10³/µl, respectively). Like the bleeding time, platelet adhesiveness was not correlated with serum creatinine or BUN

7 *Clotting time in glass and plastic tube* (Tables I and II). As for conservatively treated chronic uraemia, the clotting time was more often prolonged in plastic tube than in glass. There was no difference in the means between the acute and the chronic cases.

8. *Factor VIII* (Tables I and II). The factor VIII activity was increased in 15 of 17 cases and normal in the remaining [illegible]. The mean value was approximately the same as in chronic uraemia.

9 *Owren's P & P* (Tables I and II). P & P was normal in 13 of 18 cases, increased in 2 and decreased in 3 while in 48 patients with chronic uraemia it was normal in 21 and increased in the remaining 27 the mean being significantly lower in acute uraemia ($p < 0.005$). One of the patients with acute uraemia and increased P & P (no. 4) had had eclampsia. None of the three patients with low P & P (nos. 3 6, 14) had simultaneously thrombocytopenia and reduced values for factor VIII, factor V and fibrinogen. In two of them (nos. 6, 14) liver function tests were performed and revealed signs of hepatocellular damage.

10. *Factor V* (Tables I and II). The values found for factor V were normal in 8 of 18 cases,

increased in 4 and decreased in 6. The corresponding figures for 49 cases with chronic uraemia were normal in 34, increased in 11 and decreased in 4. It is clear from Table I that the decrease of factor V in 3 cases (nos. 1., 13, 14) was substantial. Of these patients one (no. 14) who was treated with peritoneal dialysis, had, as mentioned, also a low P & P and showed signs of impaired liver function, and in one (no. 13) there was a mild thrombocytopenia but no decrease of factor VIII, P & P or fibrinogen.

11 *Fibrinogen* (Tables I and II). The fibrinogen was increased in 14 of the patients. The results agreed largely with what we found for chronic uraemia. There was a nearly significant positive correlation with the α_2-globulins ($p < 0.05$).

1.. *Plasminogen* (Tables I and II). The plasminogen was normal in all cases except one (no. 3). This patient also had a subnormal P & P and thrombocytopenia. In another (no. 4) the plasminogen must be regarded as relatively low because the patient recently had been pregnant. She also had a slight thrombocytopenia. The mean plasminogen level was almost significantly lower than that found in chronic uraemia ($p < 0.02$)

13 *Urokinase inhibitors* (Tables I and II). The content of inhibitors of plasminogen activation was increased in all cases except one. The mean was almost significantly higher than in chronic uraemia ($p < 0.05$).

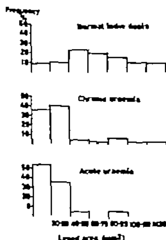


Fig. 1 Fibrinolytic activity measured as lysis of resuspended euglobulin precipitate on unheated fibrin plates in normal individuals and in cases with acute uraemia, compared with that in conservatively treated chronic uraemia.

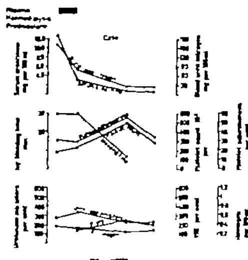


Fig. 2. Follow-up of serum creatinine, blood urea nitrogen, Ivy bleeding time, platelet count, platelet adhesiveness, factor VIII, fibrinogen and urokinase inhibitors in case 1

14 α_2-*macroglobulin* (Tables I and II). Of 10 patients examined the value was normal in five, increased in one (no. 1) and subnormal in four (nos. 2, 4, 6, 8). Patient 1 was a 13-year-old girl, so that the value must be regarded as normal for her age. The patient with the lowest value had septicaemia with impaired liver function

15 *Thrombin time* (Tables I and II). A prolonged thrombin time was found in 8 of 15 cases (in 16 of 50 with chronic uraemia). The patients with a prolonged thrombin time had numerically but not significantly higher values for fibrinogen than those with a normal thrombin time (mean $\pm$ S.D = 0.64 ± 0.24 and 0.55 ± 0.18 g 100 ml, respectively $0.3 < p < 0.4$). Fibrinolytic split products in serum were determined in 12 cases. Nine of them had measurable amounts (1.0–8.4 mg/100 ml) and one patient traces of split products (these findings are further discussed in a separate paper (19)). Five of the 9 cases and the last mentioned one had prolonged thrombin time.

16 *Fibrinolytic activity* (Fig. 1). As in chronic uraemia, the fibrinolytic activity of euglobulin precipitate (and of plasma, not included in figure) was mostly low or not demonstrable. In case 15 with normal content of inhibitors of plasminogen activation some fibrinolytic activity was found.

17 *Follow-up* (Figs. 2–6). In 7 cases the examination was repeated after the uraemia had regressed. One (no. 4) of these was examined only

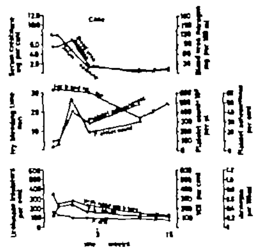


Fig 3 Follow-up of case 3

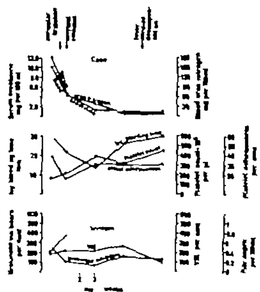


Fig 5 Follow-up of case 8. Haemodialysis given twice during the first week.

twice; on the second occasion, 10 days after the first examination, BUN was normal. The courses of those 7 cases (nos. 1 3 4 5 7 8 13) are given in Figs. 2–6.

In all cases except one the platelet count increased early. The Ivy bleeding time was primarily 30 min in 4 cases (nos. 1 3 7 8) and initially decreased with BUN and serum creatinine in these cases. Later in the course it increased again, however in two (nos. 3 8), though there were no longer any manifestations of renal insufficiency. The records contained no notes about any previous treatment with acetyl-salicylic acid or other drugs impairing platelet function, but the possibility of such treatment could not be excluded.

In two cases (nos. 3 13) fibrinogen was normal at the first examination but had increased by the following one, two days later. In these patients there was thrombocytopenia initially. One of them also had a subnormal value of P & P and the other for factor V. Factor VIII was, however, increased in both patients.

Factor VIII, fibrinogen and inhibitors of plasminogen activation decreased when the renal function became normal, but more slowly than the parameters of renal function. In three cases (nos. 1 5 7) treatment with corticosteroids had been given throughout the follow-up period and in

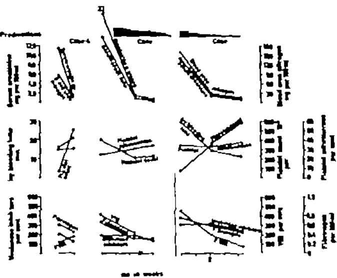


Fig 4 Follow-up of cases 4, 5 and 7

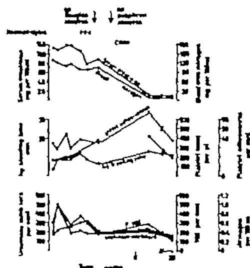


Fig. 4. Follow-up of case 13.

two others (nos. 8, 13) the course had been complicated by infections.

DISCUSSION

Acute tubular necrosis, the commonest cause of acute renal insufficiency (1), is often a complication of various conditions such as major operations, obstetric conditions and severe infections, which may by themselves lead to disorders of haemostasis (5 11). Also the treatment often given in these cases, such as repeated transfusions of bank blood and/or infusion with dextran 70, haemodialysis with heparinization, long-time treatment with broad spectrum antibiotics, corticosteroids and analgetics, may cause changes in the coagulation and fibrinolytic systems, as may complicating infections.

Chronic uraemia is a more precisely defined clinical entity than acute uraemia when the latter is due to acute tubular necrosis. In chronic uraemia the clinical picture is dominated more markedly by the impairment of renal function. Nevertheless the coagulation and fibrinolytic patterns in acute and chronic uraemia were similar in several respects. Thus both conditions were characterized by a prolonged Ivy bleeding time, decreased fibrinolytic activity increased values of factor VIII, fibrinogen and inhibitors of plasminogen activation. Thrombocytopenia, which was found to be rare in our cases of chronic uraemia, was more common in acute uraemia, while there was no difference in the number of adhesive platelets. Nor was P & P decreased in any of the cases with chronic uraemia.

The Ivy bleeding time was more often prolonged in acute than in chronic uraemia and did not vary with the severity of renal insufficiency in the acute condition. This may possibly be explained by the substances, affecting platelet aggregation, being retained to a greater extent in acute uraemia.

It has been stated that renal injury n acute tubular necrosis is caused by disseminated intravascular coagulation, precipitated by the primary disease (8 22, 38). Yet none of the present patients with acute tubular necrosis and a platelet count of less than 100 000 per μl showed the other characteristics of the coagulation pattern of disseminated intravascular coagulation, i.e. simultaneously low values for factor VIII, P & P factor V and fibrinogen. One of the other patients with a platelet count of 130 000 had low values for P & P and factor V and normal fibrinogen but an increased factor VIII activity In this case liver function was impaired, which may explain the decrease of factor V and P & P

In the five cases with glomerulonephritis the changes were largely similar to those found in most of the cases with acute tubular necrosis. Thus none of our patients with acute uraemia revealed certain signs of disseminated intravascular coagulation. It is possible that the thrombocytopenia, at least in the cases with infections, was caused by toxic medullary injury Repeated transfusions of bank blood may also cause thrombocytopenia, but none of our patients had received blood often enough to explain the decreased platelet count. The other findings in the present study and those in another investigation (19), i.e. high values for coagulation factors, low fibrinolytic activity and often fibrinolytic split products both in serum and urine, are well compatible with a reactive process and local fibrin deposition in the kidneys, which has been demonstrated in experimental and human glomerulonephritis and in acute tubular necrosis (10, 16, 21 23 36, 37). It is not known whether such deposition of fibrin is primary We feel that it may be secondary to the renal diseases and that the decrease of fibrinolytic activity may be a contributory cause.

As in chronic uraemia, the decrease of the fi-

brinolytic activity in acute uraemia probably depends on the increased content of inhibitors of plasminogen activation (13 18) and not, as previously supposed (24) on a decreased production of urokinase or other activators of fibrinolysis in the damaged kidneys.

Factor VIII fibrinogen, and inhibitors of plasminogen activation tended to decrease, when serum creatinine and BUN were normalized, but often more slowly than the parameters of the renal function. This might have been caused by corticosteroid therapy or complicating infections in five of the cases. It is also possible, however that it was due partly to persisting renal disease, although there were no signs of renal insufficiency

Like other investigators, we feel that treatment with anticoagulants may be indicated in cases of disseminated intravascular coagulation. We also feel that such treatment may be considered in cases with a local deposition of fibrin even if it is not primary because it may arrest progression of the renal disease. That anticoagulant treatment may sometimes have a good effect is supported by recent experience (15 20) In corticosteroid therapy of cases with glomerulonephritis and related conditions the primarily low fibrinolytic activity should be kept in mind, because these steroids *per se* may reduce the fibrinolytic activity (12)

ACKNOWLEDGEMENTS

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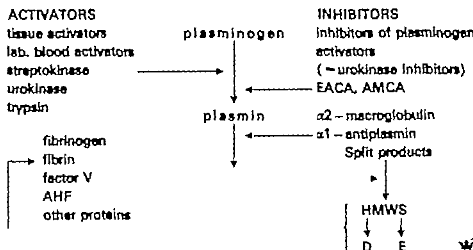
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Urinary tract haemorrhages may be caused by increased fibrinolytic activity Cyklokapron reduces or arrests fibrinolytic bleeding

In recent years fibrinolytic inhibitors have found widespread use in a number of haemorrhagic conditions, particularly in urinary tract haemorrhages and in connection with prostate surgery. Urine contains urokinase. This enzyme activates the conversion of the plasminogen present in the blood and blood clots into the proteolytic enzyme plasmin, which dissolves clots and thus sustains various types of haemorrhage in the urinary tract. Cyklokapron produces a haemostatic effect by counteracting the activity of urokinase.

The Swedish investigators, Lennart Andersson and Inga Marie Nilsson, have obtained good clinical results by administering Cyklokapron to patients suffering from haemorrhages in the upper and lower urinary tract as well as postoperative bleeding following prostate surgery. Patients suffering from haematuria as a result of general fibrinolysis were also included in the investigation. Bleeding ceased completely in all the patients in the latter group, as was the case with most of the other patients.

the fibrinolytic system



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ON COAGULATION AND FIBRINOLYSIS IN URAEMIC PATIENTS ON MAINTENANCE HAEMODIALYSIS

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Abstract. Twenty cases of chronic uraemia, on maintenance haemodialysis, have been examined in respect of their coagulation and fibrinolytic status immediately before, 18 and 42 hours after routine haemodialysis (mean duration 6.4 hours). In two of the cases the same determinations were made also after prolonged (10 hours) dialysis. The examinations before dialysis showed, as in conservatively treated cases, prolonged Ivy bleeding time, increased content of factor VIII, PAF and fibrinogen, low fibrinolytic activity and increased content of inhibitors of plasminogen activation in high frequency. After dialysis one fourth of the cases showed transient shortening of the bleeding time, and about half of the cases an increase of factor VIII, fibrinogen and inhibition of plasminogen activation, while the low fibrinolytic activity remained unchanged. The platelet adhesiveness, which was primarily low in five cases, increased in four. In both of the two cases treated with dialysis for 10 hours the Ivy bleeding time was normal 18 hours after dialysis. It is concluded that the presence of haemorrhagic diathesis in uraemia indicates an intensification of treatment with haemodialysis and that the tendency to clotting in certain cases with an external shunt is probably due largely to local endothelial damage, with release of thromboplastic substances, in combination with the generally low fibrinolytic activity in uraemia.

Uraemia is often complicated by haemorrhagic diathesis. Severe haemorrhagic complications such as gastro-intestinal bleeding, haemopericardium, subdural haematoma have been reported (1–4, 19 and others). Such a complication can be accentuated by systemic heparinization during haemodialysis (19). On the other hand, haemodialysis has been described as reducing the tendency to bleeding in uraemia (21, 31).

Treatment with haemodialysis may sometimes also be complicated by clotting in the external shunts (8, 15, 28, 33 and others).

These complications, bleeding, sometimes life threatening, and, second, clotting in the shunt which, if repeated, may limit the survival of the shunt, have drawn attention to haemostasis in patients undergoing haemodialysis because of uraemia.

In previous investigations of the coagulation and fibrinolytic systems in acute and conservatively treated chronic uraemia it was found that the picture in these two conditions is characterized, above all, by prolonged Ivy bleeding time, increased content of factor VIII and fibrinogen, decreased fibrinolytic activity and increased content of inhibitors of plasminogen activation (17, 18). Below a report is given of a corresponding investigation of cases of chronic uraemia on maintenance haemodialysis. The results are compared with those obtained in the earlier investigations, and the effect of dialysis on coagulation and fibrinolytic systems has been studied.

MATERIAL

The clinical material consisted of 20 patients, 11 women and 9 men, who had been treated with haemodialysis 3–438 times (mean 135), usually twice a week. The patients' ages, serum creatinine and blood urea nitrogen (BUN) as well as the type of renal disease and the type of shunts are given in Table I. Of the four patients, who had undergone bilateral nephrectomy and had been operated upon 11–13 months before the present investigation, two had had chronic glomerulonephritis and two chronic pyelonephritis. In all of the patients the intake of fluid as well as of sodium and potassium was restricted. All of the patients received water soluble vitamins and four of them also D-vitamin with calcium. Eight patients received antibiotics, and four were treated with dicoumarol because of recurrent clotting in the external shunt. Gammaglobulin was given regularly to five pa[…] anabolic steroids to four. Alkalinizing age[…]

Table I. *Age, aetiology of uraemia, serum creatinine and blood urea nitrogen immediately before the actual dialysis, type of shunts, number of treatments with haemodialysis in 20 cases of chronic uraemia on regular haemodialysis*

Age Mea. ± S.D.	No. of pats: Chronic glomerulonephritis	Chronic pyelonephritis	Bilateral nephrectomy*	Serum creatinine Mean ± S.D. (mg/100 ml)	BUN Mean ± S.D. (mg/100 ml)	No. of pats. with: External shunt (Quinton-Scribner)	Subcutaneous arterio-venous fistula (Cimino-Brescia)	Treatments with dialysis mean range
34.4 ± 13.8	6	10	4	15.7 ± 3.7	127 ± 40	6	14	135 (3–418)

* Two of these cases had had chronic glomerulonephritis and two chronic pyelonephritis.

[illegible] resins and antihypertensive agents were given to 15, 11 and 3 patients, respectively.

METHODS

Collection of blood. The blood was collected by the silicone technique and citrated plasma and serum were prepared as described previously (23, 29).

Coagulation studies. The following determinations were made: bleeding time, platelet count, platelet adhesiveness in whole blood, coagulation time in glass and plastic tubes, prothrombin + factor VII + factor X (Owren's P & P test), factor V, factor VIII (AHF) and fibrinogen. Unless otherwise stated, the methods described previously were used for the determinations (23). *The platelet adhesiveness* was measured by the method of Hellem (12), as modified by Cronberg et al. (7). *The fibrinogen* was determined by the technique described by Nilsson and Olow (25). Only fibrinogen values for blood collected with E-ACA are given. The *Duke and Ivy bleeding time* were measured as described by Nilsson et al. (24).

Fibrinolytic studies. The following determinations were made: fibrinolytic activity of plasma and resuspended euglobulin precipitate on unheated fibrin plates, plasminogen and inhibitors of plasminogen activation by urokinase (urokinase inhibitors). The methods described earlier (26, 27, 29) were used.

Table II. *Laboratory findings immediately before dialysis*

S = subcutaneous arteriovenous fistula. E = external shunt

Case	Age (y)	Serum creatinine (mg/100 ml)	BUN (mg/100 ml)	Platelet count (× 10³/µl)	Bleeding time (min): Duke	Bleeding time (min): Ivy	Platelet adhesiveness (%)	Clotting time (min): Glass	Clotting time (min): Plastic
1	43	9.9	153	200	4.0	30	16	12	24
2	38	12.0	140	220	4.0	30	27	6	15
3	14	13.4	96	200	—	—	29	—	—
4	60	13.8	105	220	30.0	30	14	11	24
5	42	14.0	86	256	12.0	30	19	15	27
6	41	15.9	96	279	3.0	30	39	—	—
7	53	16.2	75	140	3.0	30	19	—	—
8	19	16.7	160	344	10.0	30	18	15	24
9	24	16.8	159	200	15.0	30	35	—	—
10	60	23.0	111	200	4.0	30	39	—	—
11	27	31.4	144	260	5.0	30	20	11	30
12	34	14.9	99	249	2.5	30	23	14	27
13	26	17.7	72	376	4.0	30	32	—	—
14	21	18.0	195	220	4.0	30	29	9	27
15	20	20.1	168	100	6.0	30	20	10	26
16	23	20.1	204	100	9.0	30	20	16	33
17	46	11.1	104	500	7.0	30	28	—	—
18	30	14.1	93	140	8.0	27	32	9	1
19	23	16.2	174	200	4.0	27	31	6	24
20	43	18.3	111	200	4.0	30	27	11	17
Normal range		0.6–1.2	10–20	200–400	1–5	6–12	20–52	8–14	15–25

Haemodialysis. The patients were examined immediately before and 18 and 42 hours after a routine treatment (mean duration 6.4 hours) with haemodialysis. The dialysis was performed with disposable artificial kidney of the Alwall type (3) and a blood pump. Systemic heparinization was used. During the actual treatment ten patients received an infusion of packed red cells, 250–350 ml, eight patients analgetics containing acetylsalicylic acid in a total dose of 0.3–1.0 g and five phenothiazine preparations. Heparin was administered in a dose of 10–46 IU h kg bodyweight. T patients were examined in the same way also after prolonged (10 hours) dialysis.

Statistical analysis of data. The results obtained immediately before dialysis were compared with those obtained at examination of cases with conservatively treated chronic uraemia (with serum creatinine > 10 mg/100 ml, 23 cases) and acute uraemia (18 cases). In addition, the values obtained before dialysis were compared with one another and with age, number of dialyses before the investigation, haematocrit, concentration of haemoglobin, serum creatinine, blood urea nitrogen (BUN), serum electrolytes, serum proteins, SGOT SGPT alkaline phosphatase and total serum bilirubin. Since the Ivy bleeding time was measured only up to a maximum of 30 min, and since 17 of 19 patients had a value of > 30 min, the Ivy values were not studied for any correlation with other variables.

The differences between the values found before and on the day after dialysis were converted to percentages of the original values. The differences in platelet adhesiveness, factor VIII, fibrinogen and urokinase inhibitors were studied for any correlation with differences in serum creatinine, BUN and total protein, as well as with the number of previous dialyses, loss of bodyweight and dose of heparin. The coefficients of correlation were calculated in the usual way. Only correlations with $p < 0.05$ and of special interest are given in the text.

RESULTS

1. *Bleeding tendency.* Twelve of the 16 patients who had not received dicoumarol showed a clinical tendency to bleeding manifested by nose bleeding 6, postpuncture haemorrhage from subcutaneous arterio-venous fistulae 5, subcutaneous bleeding 3, subconjunctival bleeding 1, rectal bleeding 1 and haemothorax 1. On one occasion one of the patients bled about 1 hours after dialysis from the site of the puncture and required blood infusion.

2. *Shunt clotting.* In the six patients with a Teflon-Silastic shunt (Quinton-Scribner) clotting in the shunt occurred with a frequency of once in 35 months to 87 times in 54 months of treatment.

3. *Duke bleeding time* (Tables II and III). In 8 of 19 cases the Duke bleeding time was prolonged. No significant difference in this respect was found between the present material and the acute (4 of 18 cases) and the conservatively

Factor VIII ( )	P & P ( )	Factor V (%)	Fibrinogen (g/100 ml)	Plasminogen ( )	Urokinase inhibitors (%)	Type of shunt	Diagnosis
140	120	98	0.60	188	244	S	Chronic pyelonephritis
219	136	88	0.35	67	280	E	Chronic pyelonephritis
227	116	91	0.34	72	259	S	Chronic pyelonephritis
175	160	94	0.44	63	350	S	Chronic pyelonephritis
220	104	110	0.71	105	370	S	Chronic pyelonephritis
177	100	76	0.38	103	170	S	Chronic pyelonephritis
175	—	93	0.37	97	162	E	Chronic pyelonephritis
195	99	110	0.33	98	257	S	Chronic pyelonephritis
169	—	100	0.32	104	129	E	Chronic pyelonephritis
285	116	100	0.46	139	238	S	Chronic pyelonephritis
375	130	115	0.60	116	222	S	Chronic glomerulonephritis
—	92	98	0.36	101	181	S	Chronic glomerulonephritis
430	—	92	0.27	125	179	E	Chronic glomerulonephritis
440	136	72	0.52	163	182	S	Chronic glomerulonephritis
276	132	40	0.35	92	266	S	Chronic glomerulonephritis
123	150	70	0.34	87	246	S	Chronic glomerulonephritis
284	—	—	0.64	137	208	E	Bilateral nephrectomy
140	176	87	0.50	96	214	E	Bilateral nephrectomy
118	104	25	0.34	93	217	S	Bilateral nephrectomy
190	132	95	0.44	102	193	S	Bilateral nephrectomy
60–160	80–170	80–120	0.20–0.40	60–140	60–140		

Table III. *The occurrence of abnormal findings and means of laboratory values in present material, compared with previously investigated materials of acute uraemia (18 cases) and of conservatively treated chronic uraemia with serum creatinine of ≥10 mg/100 ml (25 cases)*

	Chronic uraemia treated with haemodialysis			Conservatively treated chronic uraemia with serum creatinine ≥10 mg/100 ml			Acute uraemia		
	Abnormal findings in			Abnormal findings in			Abnormal findings in		
	> Normal	< Normal	Mean ± S.D	> Normal	< Normal	Mean ± S.D	> Normal	< Normal	Mean ± S D
Serum creatinine			15.7±3 7			14.3±4.6			12.6±5.3 mg/100 ml
BUN			127±40			146±43			144±44 mg/100 ml
Platelet count		4 of 20	230±94		9 of 24	212±73		11 of 18	196±131 × 10³/µl
<10⁵/µl		0			1 of 24			6 of 18	
Bleeding time									
Duke	8 of 19		7.3±6.5	8 of 25		5.8±5.7	4 of 18		5.2±4.3 min
Ivy	19 of 19			16 of 23			16 of 18		
≥30 min	17 of 19			7 of 23			8 of 18		
Platelet adhesiveness		5 of 20	25 ±7		9 of 25	23±10		4 of 18	30±13 %
Clotting time									
Glass	3 of 13	2 of 13	11.2±3 2	2 of 24	0	11.8±1.8	3 of 17	0	12.6±1.9 min
Plastic	7 of 13	0	26.1±5.4	14 of 24	0	26.9±5 9	8 of 16	0	26.9±7.3 min
Factor VIII	16 of 19	0	234±95	20 of 25	0	256±119	15 of 17	0	258±120 %
P & P	8 of 16	0	125±23	10 of 24	0	124±33	2 of 18	3 of 18	103±34 %
Factor V	0	5 of 19	87 ±23	3 of 24	4 of 24	100±16	4 of 18	6 of 18	95±43 %
Fibrinogen	9 of 20	0	0.43±0.12	22 of 25	0	0.55±0.18	14 of 18	0	0.61±0.23 g/100 ml
Plasminogen	2 of 20	0	107±31	2 of 25	0	113±34	0	1 of 17	93±25 %
Urokinase inhibitors	19 of 20	0	228±60	20 of 25	0	198±68	16 of 17	0	258±93 %

treated chronic cases (8 of 25) In 3 of the 8 cases with prolonged Duke bleeding time, platelet adhesiveness was decreased.

4 *Ivy bleeding time* (Tables II and III) All 19 patients examined had a prolonged Ivy bleeding time, and in 17 it was 30 min or more. The latter figure indicates a significantly higher frequency than in the acute (8 of 18 $p<0.005$) and the conservatively treated chronic cases (7 of 23 $p<0.001$). In 5 of the 17 cases with a bleeding time of at least 30 min the platelet adhesiveness was reduced.

5 *Platelet count* (Tables II and III) In none of the patients was the platelet count less than 100 000/µl. In four it was less than 200 000 but more than 100 000. The number of patients with a count of less than 200 000/µl was significantly lower than found in acute uraemia (11 of 18, $p<0.01$).

6. *Platelet adhesiveness* (Tables II and III). Platelet adhesiveness was reduced in 5 of 20 cases, compared with 9 of 25 in conservatively treated chronic uraemia, and 4 of 18 in acute uraemia. All patients with a reduced platelet adhesiveness had an Ivy bleeding time of 30 min or more.

7 *Clotting time in glass and in plastic tubes* (Tables II and III). As in acute and conservatively treated chronic uraemia, the clotting time was more often prolonged in plastic tubes than in glass tubes. The deviations from the normal range were, however usually inconsiderable.

8 *Factor VIII* (Tables II and III). Factor VIII was raised in 16 of 19 patients and normal in the remaining 3 The frequency of increased values and the mean value were roughly the same as in acute and conservatively treated chronic uraemia.

9 *Owren s P & P* (Tables II and III). P & P was increased in 8 of 16 patients not treated with dicoumarol. Only in three cases, however were the deviations noteworthy The mean was roughly the same as in conservatively treated chronic

uraemia, but almost significantly higher than in the cases of acute uraemia ($p < 0.05$).

10. *Factor V* (Tables II and III) In five patients factor V was subnormal, but in only two was it less than 50% In these two cases P & P factor VIII and fibrinogen were normal or increased and the platelet count not below 100 000/µl. In one of them (no 19) the values for SGOT and SGPT were increased (54 and 198 U respectively). The mean value for factor V was almost significantly lower than in conservatively treated chronic uraemia ($p < 0.05$).

11 *Fibrinogen* (Tables II and III). Fibrinogen was elevated in 9 of 20 cases and normal in the remaining 11 The mean was significantly lower than in acute ($p < 0.005$) and almost significantly lower than in conservatively treated chronic uraemia ($p < 0.02$). Fibrinogen increased significantly with increasing α_1-globulins ($p < 0.01$) and decreasing albumin ($p < 0.01$), while the positive correlation with α_2-globulins was not significant ($0.05 < p < 0.1$).

12. *Fibrinolytic activity* (Fig. 1). As in acute and conservatively treated chronic uraemia, fibrinolytic activity of euglobulin precipitate (and of plasma, not included in figure) was low or not demonstrable in most of the cases.

13 *Plasminogen* (Tables II and III). The plasminogen value was normal in 18 patients and increased in two. In these two the fibrinogen was increased, and the fibrinolytic activity low in one and not demonstrable in the other

14 *Urokinase inhibitors* (Tables II and III). An increased content of the urokinase inhibitors was found in all cases except one, in which it was normal. This patient had normal fibrinolytic activity and a normal content of fibrinogen. A significantly negative correlation was found with the total protein and albumin ($p < 0.01$) while the positive correlation with α_1-globulins was not significant ($0.05 < p < 0.1$).

15. *Coagulation status—number of dialyses, cause of uraemia and type of shunt* No significant correlations were found between the coagulation-fibrinolytic status and the number of treatments with dialysis or aetiology of uraemia. In cases with external shunt there was an almost significantly ($p < 0.05$) lower mean value for urokinase inhibitors than in those with subcutaneous fistula, but otherwise there was no difference between these groups.

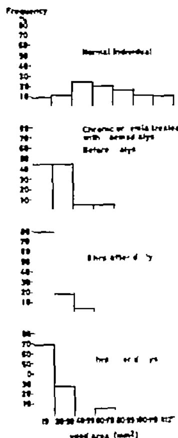


Fig 1 Fibrinolytic activity measured as lysis of suspended euglobulin precipitate on unheated fibrin plates in normal individuals and in chronic uraemia treated with haemodialysis, immediately before, 18 and 42 hours after haemodialysis.

16. *The effect of haemodialysis on coagulation and fibrinolytic status* (Figs. 1–4). The loss of weight as measured 18 hours after an ordinary dialysis varied from 0.9 to 5.0% (mean 3.5%) of the weight before. One patient showed no reduction in serum creatinine and a fall of 10% of BUN In the other patients the serum creatinine fell 15–39% and the BUN 9–46% (means for the entire series 27 and 35% respectively).

Duke bleeding time was shorter on the day after dialysis in 5 of the 8 cases with a primarily increased value, and the Ivy bleeding time in 4 As for the Ivy time, the fact that measurement was discontinued at 30 min might have masked a reduction in some cases and might also explain why a shortening of the bleeding time to less than 30 min in 3 cases was not observed until the second day after dialysis.

Fig 2

Fig. 3

Figs. 2–3 Blood urea nitrogen, serum creatinine, Duke and Ivy bleeding times, platelet adhesiveness, factor VIII, P & P factor V fibrinogen, urokinase inhibitors and plasminogen before, 18 and 42 hours after routine haemodialysis (mean 6.4 hours) in 11 cases with subcutaneous arteriovenous fistula (Figs. 2, 3 *a*) and in 6 cases with external shunts (Fig. 3 *b*). Numerals denote patients numbers given in Table II.

In 4 of the 5 cases in which platelet adhesiveness was reduced before dialysis an increase was noted on the day after dialysis. In 3 cases a decrease occurred on the day after dialysis and in 2 on the second day

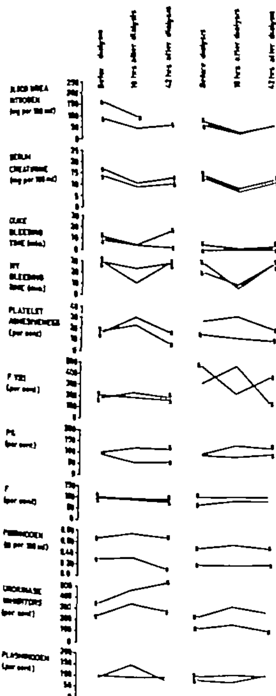


Fig. 4. Findings in two patients after 7 (*a*) and 10 (*b*) hours dialysis.

The mean value for urokinase inhibitors had increased significantly ($p < 0.01$) on the day after dialysis and that of factor VIII almost significantly ($p < 0.05$). There was also some tendency

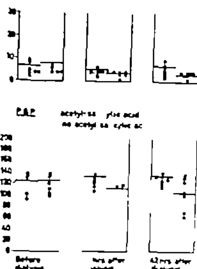


Fig. 5. Changes in the Duke bleeding time after haemodialysis in patients given and not given red cell mass infusions during dialysis and changes in P & P in patients given and not given acetylsalicylic acid.

for factor V and fibrinogen to increase while P & P, plasminogen and the fibrinolytic ... it remained largely unchanged.

Fig. 3 *b* shows that the cases with external shunts did not deviate substantially from those with an internal fistula as regards changes in coagulation pattern after dialysis.

The investigation showed no significant correlation between the differences in the coagulation fibrinolytic status and number of dialyses, dose of heparin, duration of dialysis, or reduction in weight, serum creatinine or BUN.

One (no. 8) of the two patients examined after a prolonged (10 hours') dialysis (Fig. 4) was found to be in a better clinical and biochemical condition than at the previous examination. In both cases the Ivy bleeding time became normal on the day after dialysis, but on the following day it was again more than 30 min. Otherwise no appreciable differences were found in the coagulation and fibrinolytic status compared with that after the shorter dialysis treatment.

Effect of blood infusion and drugs on coagulation fibrinolytic status after dialysis (Fig. 5). In the 10 patients given packed red cells during haemodialysis the Duke bleeding time 42 hours after dialysis was almost significantly shorter than

in those who had not received blood ($p<0.05$), though the value before dialysis had been numerically higher. Fig. 5 also shows that in cases in which acetyl-salicylic acid was given during dialysis, the P & P value fell, and on the second day after dialysis it was almost significantly lower than in cases in which acetyl-salicylic acid had not been given ($p<0.05$). No other effects of infusion of packed red cells or drugs on the coagulation status were observed.

DISCUSSION

From a biochemical point of view chronic uraemia treated with haemodialysis is not the same as conservatively treated uraemia, partly because of the varying dialysability of substances retained in uraemia and partly because substances, normally retained, such as amino acids, may be lost during dialysis. Despite this difference the investigation showed that the coagulation and fibrinolytic pattern in patients treated with haemodialysis closely resembled that found in non-dialysed cases. In both conditions prolonged Ivy bleeding time, increased content of factor VIII, P & P and fi- low fibrinolytic activity and high con- of inhibitors of plasminogen activation were common. But the Ivy bleeding time was prolonged more often in patients subjected to dialysis, even though the retention of urea tended to be less pronounced. Since the patients had no thrombocytopenia, prolongation of the bleeding time was presumably due to impaired adhesion and aggregation of platelets. In some cases with a prolonged Ivy bleeding time, however, platelet adhesiveness was normal. This has also been observed in other investigations, both by Borchgrevink's (5) and Salzman's (9) methods.

Clinically platelet function has been found to vary with the severity of uraemia (5 9), but neither in vitro nor in vivo has urea in earlier investigations been found to impair the function of normal platelets (5 6, 13 14 30, 31). Eknoyan et al. (9), however, recently found that prolonged experimental azotaemia (10 hours or more) in man provoked by repeated administration of urea by mouth, was accompanied by a reduction of platelet adhesiveness.

That the present cases of chronic uraemia on maintenance haemodialysis despite a numerically lower degree of uraemia, as measured by BUN more often had a prolonged Ivy bleeding time than conservatively treated cases may perhaps be explained by the uraemic intoxication having existed longer and/or by the substances that are toxic for the platelets having been dialysed off less readily than urea.

Increased amounts of factor VIII, fibrinogen and of urokinase inhibitors are seen in most reactive processes and the levels observed in uraemia are probably also caused by reactive processes due to primary disease or to complications.

The tendency to lower fibrinogen content in patients on maintenance haemodialysis than in conservatively treated cases was probably not due to fluid retention, since no apparent difference was found for other factors in the coagulation and fibrinolytic systems. In conservatively treated chronic uraemia with varying severity of renal insufficiency we have previously found that fibrinogen first increased and then decreased with increasing serum creatinine, which, judging from the serum protein values, did not appear to be an effect of haemodilution (17). Ljungqvist (20) has found that fibrinogen synthesis decreases with increasing renal insufficiency It is possible that the lower values for fibrinogen in dialysed than in non-dialysed cases were due to an impaired synthesis capacity even though there was no difference in serum creatinine and that the longer duration of renal insufficiency in the patients on regular dialysis may be of importance in this respect.

As in the non-dialysed cases, the low fibrinolytic activity is probably due to the increased content of inhibitors of plasminogen activation (17) and not to decreased production of activators of plasminogen.

Shunt clotting has been ascribed by some authors to hypotension and local factors such as technically unsatisfactory surgery compression, cold, infections and vascular changes (8 16, 28 33), and by others to an increased content of coagulation factors (10). With Salzman's method Andersson and DePalma (4) found a significant increase of platelet adhesiveness during dialysis and assumed that this may contribute to post dialytic clotting. The only difference in coagulation and fibrinolytic status between cases with external and internal shunts in our series was a tendency to lower content of inhibitors of plasminogen activation in the former These observa-

sons do not suggest an increased disposition for clotting in cases with an external shunt compared with those with an internal fistula.

Glasban and Walker (11) examined histological sections of the venous wall in Quinton-Scribner shunts and found damaged endothelium and progressive fibrin deposits poor in platelets. Like these authors, we believe that the release of thromboplastic substances in endothelial injury in combination with the generally low fibrinolytic activity in uraemia contributes to the clotting in external shunts.

The increase of coagulation factors and of inhibitors of plasminogen activation found in some cases after dialysis may be due to different factors, such as haemoconcentration, reaction to post punctional haematoma or transient bacteriaemia. Only in a few cases did the Ivy bleeding time become shorter after an ordinary dialysis, and in only one did it become normal, although the effect of dialysis on the urea values was often acceptable. But in the two cases dialysed for 10 hours the Ivy bleeding time became normal on the day after dialysis. In view of the latter observation and of the good effect of haemodialysis on the bleeding tendency observed by Stewart and Castaldi (31), the development of haemorrhagic diathesis, like polyneuropathy (2), should be regarded as an indication for more intense treatment with haemodialysis in cases of chronic uraemia.

In cases in which an unexpected decrease of platelet adhesiveness occurred after dialysis, technical errors and drugs could not be excluded as possible causes.

The tendency to shortening of the Duke bleeding time noted 42 hours after dialysis in cases treated with infusion of packed red blood cells might perhaps be explained by the infusion having caused an increased availability of ADP. The reason why the P & P values successively fell after dialysis in patients who had received acetyl salicylic acid may perhaps, be explained by the assumption that the substance, though dialysable, interfered with the synthesis of the prothrombin complex.

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POST MORTEM CORONARY ARTERIOGRAPHIC, CLINICAL AND ELECTROCARDIOGRAPHIC FINDINGS IN 80 PATIENTS INVESTIGATED WITH CORONARY ARTERIOGRAPHY

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Abstract. A coronary arteriographic investigation has been made of 492 patients, most of whom had different cardiac diseases. Eighty patients died during an observation period from 1 / up to about 7 years. In 45 patients post mortem investigation was made. Comparison between arteriographic and post mortem findings in the arteries and the myocardium, as well as the study of other clinical facts at or before the time of investigation, led to the following conclusions.

1. A coronary arteriogram of good quality gives reliable picture of the intraluminal condition in the big predominantly epicardial coronary arteries.

2. An occlusion or critical obstruction of the coronary arterial stems are in the majority of hearts combined with myocardial disease in the form of old infarction scar or local, patchy fibrosis.

3. Coronary arteriography is of good help to demarcate group of patients with diffuse non-atherosclerotic, myocardial disease

4 Early changes of atherosclerotic coronary artery disease seem to be rather evenly distributed among the big coronary arteries. In the living patients the majority of severe obstructions are present in the right and the left descending artery. This distribution is probably due at least partly to the selection of patients in the study

5. Roughly half of all myocardial infarctions can be clinically traced.

6. The results emphasize that angina pectoris, clinical picture of myocardial infarction and abnormal Q waves in the ECG are not synonymous with coronary heart disease. This may be particularly true in hospitalized patients, who may overrepresent non-atherosclerotic heart diseases, leading to symptoms and signs shared by the atherosclerotic, coronary heart disease.

Coronary arteriography visualizes the large, predominantly epicardial coronary arteries in life. By exclusion of obstructive arterial disease with an angiogram, non-atherosclerotic cardiac diseases can be diagnosed in life. The natural history both of coronary artery disease and of other arterial diseases can thus be studied. In selected patients a coronary arteriogram may be helpful in pre operative or differential diagnostic evaluations.

After period dominated by methodological refinement there are at present two main objectives in coronary arteriography disregarding its use in the clinical work on the individual patient. One is to test its validity and establish correlations to clinical, anatomical and physiological data, the other to use the method in the scientific study of problems related to cardiac and vascular diseases. The present study is mainly concerned with the first of these objectives.

METHODS

Coronary arteriographic data

A semiselective technique as used with contrast injection through an end-loop catheter, with end-hole and several side-holes, positioned in the aortic bulb. Exposures were made successively in two planes using big film exchanger. Details of the method were described by Paulin (13).

The arteriographic findings were recorded according to Paulin (13). Emphasis was placed on the following factors: (*a*) Technical data and quality (*b*) Anatomy of the arteries. (*c*) Pathology with special reference to the distribution and degree of arterial obstructions and presence of collaterals.

Intraluminal pathology was classified:

A. No abnormality

B Vessel wall irregularities.

C. Stenosis without contrast filling delay beyond the stenosis.

D. Stenosis with contrast filling delay beyond the stenosis.

E. Occlusion.

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Table I. *Clinical diagnoses in the total series of 492 patients and in the 80 deaths at the end of the observation period*

As some patients had more than one cardiac diagnosis the sum will amount to more than 100%

Clinical diagnoses	All patients No.	%	Mean age (y.)	All deaths No.	%	% of all with the same diagnosis	Mean age (y.)	Cardiac deaths
Coronary heart disease	239	49	49.9	51	64	21	49.1	44
Peri- and/or myocarditis	14	3	35.1	1	1	7	32.0	0
Cardiomyopathy	40	8	40.5	10	13	25	38.4	8
Arrhythmia	76	15	45.7	11	14	14	50.3	7
Valvular or congenital heart disease	43	9	47.4	10	13	23	50.1	10
Arterial hypertension	109	22	51.5	18	23	17	52.6	14
Cardiac neurosis	31	6	45.4	1	1	3	46.0	0
Unclassifiable heart disease	15	3	40.2	1	1	7	46.0	1
N cardiovascular diagnosis	54	11	44.5	1	1	2	42.0	1

Clinical data

All patients were admitted to the hospital before the angiography for clinical investigation. All previously recorded medical facts concerning the patients were collected. The pertinent clinical data were entered on punch card, paying regard to the following factors:

1. Previous clinical diagnoses of importance.

2. Previous clinical myocardial infarctions diagnosed from the following criteria: (*a*) Development of abnormal Q waves. (*b*) Infarction pain (see below) and abnormal serum transaminase titre and/or development of abnormal ST T changes in the ECG. (*c*) Development of abnormal ST T changes and abnormal serum transaminase titre. (*d*) Suspicion of an infarct not fulfilling the criteria a–c was recorded separately

3 The history of chest pain was analysed in either of three ways: (*a*) From special interview before the arteriography according to a special scheme by one of our team—158 patients. (*b*) From special interview at a follow-up examination at least several months after the arteriography by one of our team—57 patients. (*c*) From the medical record before the arteriography—282 patients.

The following pain classification was used: (*a*) *Typical anginal pain* with precordial or retrosternal localization, with duration of more than momentary and less than half an hour and with positive relation to physical effort. (*b*) *Infarction pain* with retrosternal or precordial pain at rest lasting for at least one hour and connected with some general physical or somatic reaction like anger, paleness or weakness. (*c*) *A chest pain syndrome* similar to anginal pain either with typical localization, but lacking one or both of the other two characteristics, or with atypical localization but related to effort. (*d*) *Chest pain* similar to anginal pain where it as not possible to demarcate the exact nature of the pain experience in a confusion of multiple obscure symptoms among obviously psychoneurotic patients. (*e*) *Chest pain* similar to anginal pain but so vaguely and indifferently described that it seemed possible that the symptom had been suggested to the patient.

4. Function group classification according to the recommendation of the New York Heart Association (3).

5 Present and previous blood pressure level judged by the cuff method.

6. Present and previous heart rhythm.

7 Physical findings on the heart.

8. The clinical diagnoses at the time of angiography Regard was paid to the arteriographic findings, but no patient as diagnosed as coronary heart disease only from findings on the arteriogram.

Laboratory data

1 Present and previous blood lipid levels were collected.

2. Radiographic heart size in horizontal position was measured according to Kjellberg et al. (11 12). In some patients it was also measured in standing position according to Jonsell (9).

3 The electrocardiographic findings at the time of investigation were collected on punch card. In all patients the following leads were recorded at rest: I, II, III, aVR, VL, VF CR, In the majority of patients w also made an exercise test on bicycle ergometer according to Sjöstrand (17) and Wahlund (20).

Mortality of patients

The patients have been followed after the angiography with regard to mortality until January 1, 1968. The longest observation time has been about 7 years and the shortest in the present report 18 months.

As the patients were referred from all over Sweden, it was impossible to perform special post-mortem examination of the heart except in some cases. In the remainder data have been collected from hospital records and reports from the post-mortem examinations.

When the patient died at home and no post-mortem examination was performed, the cause of death was classified as sudden cardiac death on the death certificate.

Table II. *Mortality related to time after arteriography for all 80 deaths*

Groups with different observation time include living patients and also those who died before the actual observation time

Observ. time after arteriography (mo.)	No. of deaths	% of all with the same observ. time
1–12	33	7
13–24	14	3
25–36	12	3
37–48	10	3
49–60	6	2
61–72	4	3
73–84	1	2

The special post mortem analyses has been confined to judgement of the myocardium and the condition of the main coronary arteries, the right coronary, the left main, the left descending and the left circumflex artery.

MATERIAL

Between 1961 and 1965 coronary arteriography was performed on 492 patients. During the observation period 80 of these patients died and on 45 of them a post-mortem examination was made. The clinical diagnosis in the total series and for all patients who died are listed in Table I.

A diagnosis of *cardiomyopathy* was made for patients fulfilling certain criteria. They presented with chronic cardiac symptoms from dyspnea or arrhythmia and had signs of left ventricular enlargement but, irrespective of the previous criteria, had electrocardiographic abnormalities in the QRS or ST-T segments not explained by digitalis therapy. They had no clinical signs of coronary heart disease, pericarditis, myocarditis, valvular disease, congenital heart disease or hypertension.

A diagnosis of *unclassifiable heart disease* was made when the patients did not fulfil any of the other diagnostic criteria of heart diagnosis but had, for example, only radiographic heart enlargement or bundle branch block.

The dominating cause of death in the total series was cardiac (infarction, heart failure or sudden death). Thus 44 of the 51 patients with coronary heart disease, 8 of the 10 with cardiomyopathy, 7 of the 11 with arrhythmia, 10 of 10 with valvular or congenital heart disease and 14 of 18 with arterial hypertension died from cardiac causes. Malignant tumours, uremia, infection, suicide or accidents accounted for the other deaths (Table I).

RESULTS AND COMMENTS

Angiographic quality

Among the 80 patients it was unsatisfactory and uninformative in 5 (6 %), unsatisfactory but informative in 20 (25 %), satisfactory in 17 (21 %) and excellent in 38 (48 %). Thus in about 70 % the quality was satisfactory or excellent and in 5 patients a failure. These 5 patients were excluded from further analysis in this study.

Mortality and complications from the arteriography

In two patients complications from the angiography might have influenced a successive fatal outcome, but there was no immediate fatal complication.

Patient 104. A myocardial infarction caused rupture of the septum with a ventricular septal defect and severe cardiac failure. Open heart surgery was deemed necessary for survival. An uneventful coronary arteriography was made, whereupon the loop-catheter was forwarded into the left ventricle in order to perform a ventriculography. The patient then became acutely and permanently hemiplegic, probably due to dislodgement of endocardial thrombotic material. He died

Table III. *Clinical diagnosis, cause of death and post mortem findings in 9 patients dying during the first two months after arteriography*

Day of death after angiography	Pat. no.	Clinical diagnosis	Cause of death	Post mortem findings
2	140	Cor. heart dis.	Sudden death	—
4	415	Mitr. valv. dis.	Sudden death	—
13	18	Cor. heart dis.	Sudden death	Cor. art. dis. and patchy fibrosis
27	116	Cor. heart dis.	Art. embolism	Old infarct + art. embolism
36	200	Cor. heart dis.	Pulm. edema	Old infarct
52	144	Cor. heart dis.	Myoc. infarct	Old infarctions, fresh infarct
53	156	Cor. heart dis.	Myoc. infarct	—
55	507	Cor. heart dis., uremia	Uremia	Cor. art. dis. and patchy fibrosis + nephritis
59	164	Cor. heart dis.	Sudden death	Old infarctions + fresh infarct.

Table IV *Arteriographic pathology related to post-mortem examination (PM) of the coronary arteries in 45 patients*

Occlusions

Occluded artery	Arteriographic occlusions	PM as arterio-graphy	PM tight stenosis	PM without stenosis	PM not specified
Right	16	13	3	0	0
Left main	0	0	0	0	0
Left desc.	6	5	0	0	1
Left circ.	3	3	0	0	0

Tight stenoses ith contrast delay

Stenosed artery	Arteriographic stenoses	PM as arterio-graphy	PM occlusion	PM without tight stenoses	PM not specified
Right	2	2	0	0	0
Left main	0	0	0	0	0
Left desc.	9	2	4	0	3
Left circ.	3	1	2	0	0

about one year later from cardiac arrest. The post-mortem investigation revealed a ventricular septal defect, severe arterial disease and a cerebral *malacia*.

Patient 116 This patient had been seriously ill for five years with congestive heart failure, at one period complicated by shock. No diagnosis had been made and a coronary angiogram was performed. During the following 24 hours the patient started to develop migrating arterial oc clusions. The patient died four weeks later The post-mortem examination showed arterial throm bosis and emboli in multiple areas. It is possible (6 7) that the catheterization procedure may have instigated or promoted the thrombotic process.

When mortality is related to observation time after angiography it was constant beyond the first year when it was highest (Table II) The mortality during the first year was highest dur ing the first two months.

During these two months 9 patients died (Table III). All 8 patients with coronary heart disease were in an advanced stage of their disease and all in whom a post-mortem examination was made had widespread secondary myocardial damage.

The higher mortality during the first year of observation is best explained by the more ad vanced stage of disease at the investigation of these patients compared with the whole series.

Comparison between arteriographic and post mortem findings

When the results of the post-mortem examination are compared with the angiographic findings, the agreement regarding occlusions and severe stenoses is excellent (Table IV)

In the 10 patients in whom the coronary angiography did not reveal any intraluminal pathology (Table V), the post-mortem examination did not show any obstructive changes either

Table V *Arteriographic pathology related to pathological myocardial anatomy in all 45 patients with post-mortem investigation*

Within parentheses are the 14 patients who had history of clinical infarction

Coron. arteriographic pathology	Total no. of pats.	Pats. with infarct. scar	Pats. with local fibrosis	Pats. with fresh infarct.	No myocardial pathology
No pathology	10 (1)	0	0	0	10 (1)
Irregularities	4	0	0	1	3
Stenosis without delay	5 (2)	3 (1)	0	1	1 (1)
Stenosis with delay	5 (2)	3 (1)	0	2 (1)	0
Occlusion	21 (9)	17 (8)	2	2 (1)	0

When the myocardial alterations as recorded at autopsy are related to the findings at arteriography (Table V), 19 of 21 patients with occlusion had old secondary myocardial disease corresponding to the occlusion. The two remaining patients had a recent infarction but the possibility of old changes superimposed by the infarction cannot be excluded.

In contrast the 10 patients with normal coronary arteries showed no signs of secondary coronary myocardial damage at autopsy.

Of the 14 patients who had a clinical history of myocardial infarction before the angiography (Table V), 10 revealed old myocardial scars at post-mortem examination. Two had fresh infarcts corresponding to the occluded artery while in 2 patients no pathoanatomical verification of an old infarct was made. One had normal arteries, the other moderate stenosis according to the angiogram. At the post-mortem examination the first patient had aortic stenosis without coronary obstructions and the other moderate atherosclerosis in the coronary arteries with multiple non-occlusive stenoses.

In the total series 40 patients were diagnosed as cardiomyopathy. Ten of them died and 5 had a post-mortem examination. In all 5 a non-occlusive angiogram was a prerequisite for the diagnosis and none had any obstructions. Only a small degree of atherosclerosis was found in the coronary arteries post mortem. Four patients had diffuse myocardial fibrosis shown microscopically and one had massive myocardial infiltration of amyloid material. This was part of a generalized amyloidosis which had been diagnosed clinically. Two of the 5 patients had intracardial thromboses.

Comments. The present study confirms the validity of determining obstructive lesions in the epicardial coronary arteries from the coronary arteriogram. In all arteries where it was possible to compare the angiogram with post mortem findings, both radiologist and pathologist found the same severe obstructions. The functional difference between an occlusion and a severe obstruction as defined by us is probably not great. Differences of opinion between pathologist and radiologist regarding differentiation between severe obstruction or total occlusion may occur due to the common development of new vascular channels through the area of previous thrombosis (3). The investigation during life is probably correct, as the arter[illegible] terial pressure[illegible]

An arteriogr[illegible] struction in a m[illegible] teries may give [illegible] myocardial dam[illegible]

Allison et al. (1[illegible] by Schlesinger[illegible] with one or m[illegible] nary artery or [illegible] cardial necrosis[illegible] terial occlusion[illegible] in 11 hearts had[illegible] farction In rela[illegible] of these hearts d[illegible] infarction elsewhe[illegible] obstructions o[illegible] concluded that t[illegible] sions are accomp[illegible] Snow et al. (18) ar[illegible]

The results of B[illegible] at variance with th[illegible] with old occlusions [illegible] only in 15 and 6 wer[illegible] old infarction. Consequentl[illegible] occlusion without infarct[illegible] co[illegible] due to development of interco[illegible]

This difference in results w[illegible] techniques is probably due not only to [illegible] modes of interpretation but rather to large differences in selection of the investigated hearts.

This study supports the opinion of others who have compared their arteriographic findings with post mortem dissection of the coronary arteries (10). An arteriogram of good quality gives a reliable picture of obstructive lesions in the epicardial coronary arteries, where the atherosclerotic process mainly takes place. Our data also show that an occlusion or a critical obstruction in a main stem of the coronary arteries in almost all patients is connected with myocardial damage in the form of infarction scar or patchy fibrosis. An arteriogram lacking obstructions signifies, on the other hand, that there are no such coronary myocardial changes to be found.

Cardiomyopathy is a term which usually has been assigned to a group of myocardial diseases characterized by diffuse non-infectious myocardial pathology or fibrosis with or without concomitant inflammatory reaction. The etiology is not uni[illegible] sometimes obscure, and the microscop[illegible]

Table VI. *Distribution of arteriographic pathology in the right, left main, left descending and left circumflex artery of 62 patients in whom all arterial stems were visualized in detail*

For each degree of pathology the distribution is also given in %

	Right		Left main		Left desc.		Left circ.	
	No.	%	No.	%	No.	%	No.	%
No pathology	15	20	31	41	13	17	17	22
Irregularities	21	26	25	31	11	14	24	30
Stenosis without delay	8	21	5	13	13	[illegible]9	14	37
Stenosis with delay	4	18	1	5	12	55	5	23
Occlusion	14	45	0	0	15	48	2	6

picture is often unspecific, with such exceptions as amyloidosis or sarcoidosis.

According to James (8) primary involvement of the small coronary vessels *may constitute the* main pathology. Sometimes this may be connected with a systemic form of disease.

The present technique for coronary arteriography cannot visualize the small intramyocardial vessels. Some typical arteriographic changes may possibly indicate the presence of diffuse cardiomyopathy (21).

Previous comparisons between in vivo arteriographic and post mortem myocardial findings are scarce. The present series is rather small and the post-mortem examinations not too detailed. This emphasizes the need for more comparative studies which may add further information about what conclusions may be *drawn from a* coronary arteriography.

Distribution of arteriographic pathology in the coronary arteries

The distribution of intraluminal changes in the main coronary arteries—the right, left main, left descending and left circumflex artery—was studied in the 62 patients in whom all main stems could be judged in detail in the angiogram (Table VI).

Occlusions were uncommon in the left circumflex artery. The left main artery contained no occlusion at all. Combining occlusions and near occlusions as an entity of severe obstruction, the same result appears. The right and left descending arteries were the site of the majority of severe obstructions, while obstructions of the left circumflex and left main arteries were rare.

The distribution of lesser degree of pathology was different. Moderate stenoses were equally often located in the left main and circumflex as in the other arteries. Wall irregularities were slightly more often found in these two (61%) than in the right or left descending artery.

Comments. *That intraluminal changes in the* left main artery are uncommon may be due to the small length of this artery in comparison to the others, but may also have some hemodynamic *explanation* (19).

Proudfit et al. (14) in 627 clinical angiograms, found severe obstructions in the right and left descending artery in 39 and 38% respectively, left circumflex in 20% and left main artery in only 3%.

Gensini and Buonanno (4), in 100 clinical angiograms from patients with coronary heart disease found the following distribution of stem occlusions: right 51%, left descending 36%, left circumflex 13%. The left main artery was not occluded at all.

The earlier results of post-mortem investigations (15, 16) have been similar. Most notable is that in these series the left circumflex artery was occluded more often (20 to 30%).

These studies thus indicate that severe obstruction is more common in the right and left descending arteries. These arteries are the site of between 30 and 50% of severe stem obstructions, while the left circumflex artery has fewer obstructions. Yet this artery seems to be more often *occluded post* mortem than in the *living* state. Occlusion of the left main artery is uncommon in the living patient.

A hypothesis that possibly might explain this distribution of severe pathology is based on the varying ability to form collateral vessels. The left circumflex artery *supplies a big part of* the left ventricle. In the lateral part of that ventricle conditions are unfavourable for development of collaterals *from other arteries. Severe obstructions* in or occlusions of the circumflex artery might thus lead to acute death more often than other obstructions.

Patients with severe obstructive coronary artery disease who are submitted to coronary arteriography belong to a selected group because

they have survived a severe obstruction. The patients not surviving severe obstructions comprise those studied post mortem, where severe obstruction of the left circumflex artery seems to b more common than in vivo (15) Published post mortem materials are, however also selected in that the patients have been admitted to hospital, indicating that some time has elapsed from the time of occlusion and death. A post mortem study of patients with coronary heart disease dying suddenly outside hospital has therefore been initiated.

Arteriographic and post mortem pathology related to clinical signs of coronary heart disease

Anginal pain occurred in several patients without arterial obstructions and also lacking clinical diagnosis of coronary heart disease (Table VII). These patients had aortic valvular disease, arterial hypertension or cardiomyopathy The post mortem examination confirmed the clinical diagnosis and demonstrated the absence of significant coronary artery stenoses.

In all, 45 patients had one or more arterial tight stenoses or occlusions. Thirty-nine of these (87%) had anginal pain and 19 (42%) had had infarction pain.

One patient had infarction pain without any arterial pathology She had aortic valvular disease and the post-mortem examination revealed unobstructed coronary vessels and no myocardial necrosis.

Clinical signs of coronary heart disease as well as a history of old infarction appeared in the group of patients with moderate arterial stenoses.

Table VII *The relation between arteriographic pathology coronary pain and diagnosis in 75 patients*

MI myocardial infarction.

Coron. arteriographic pathology	Total no.	Angina pectoris	Infarction pain	Clinical diagnoses: Cor heart dis	MI
No pathology	14	4	1	0	1
Irregularities	9	4	0	0	0
Stenoses without delay	7	5	1	6	2
Stenosis with delay	10	10	5	9	4
Occlusion	35	29	14	35	16

Table VIII *Coronary arteriographic pathology related to abnormal Q waves in the ECG at rest Q waves of type I II and III refer to the classification in the Minnesota coding system*

Coron. arteriographic pathology	No. of pats.	Pathol Q	Q: I	II	III	Pathol Q ( )
No pathology	14		0	0	2	14
Irregularities	9	3	0	1	2	33
Stenosis without delay	7	0	0	0	0	0
Stenosis with delay	10	3	1	0	2	30
Occlusion	35	18	6	5	7	51

Forty-four of 45 patient with a severe obstruction had other clinical evidence than the result of angiography indicating coronary artery disease. Only 20 (44%) had a clinical history of myocardial infarction Similarly only 16 of 35 patients with at least one occluded artery (46%) had a history of clinical infarction.

Abnormal Q waves at rest occurred in 5 patients of 23 without arterial obstructions (Table VIII). They had either cardiomyopathy or aortic valvular disease diagnosed both clinically and at post-mortem examination None showed any infarction scar in the myocardium.

Fifty-one % of patients with occlusion had abnormal Q waves, figure similar to the occurrence of infarction pain in the same patients. Combining patients with occlusion and tight stenoses in one group, 47% had abnormal Q waves in the resting ECG

When angiographic pathology was related to abnormal ST T-changes at rest, it was not possible to discriminate patients with obstructive coronary artery disease.

Comments. Anginal pain, clinical picture of infarction and abnormal Q waves are thus not synonymous with coronary heart disease. I large scale epidemiological studies abnormal Q waves have sometimes been used as evidence of coronary heart disease. This has been inferred on the basis of the overwhelming predominance of coronary artery disease as cause of myocardial scarring. As shown here, however this may not be justified even in countries like the USA or Western Europe among patients admitted to a hospital.

Future studies will show whether the Minnesota code will help to differentiate between coronary heart disease and other types of myocardial diseases as it did in our series, in which the non-coronary patients had Q waves of type II and III but not of type I.

It was stated above that about half of all infarctions are clinically visible. Evidence was also presented that most total occlusions were connected with old myocardial scars. About half of all patients with occlusions ought thus to have clinical signs of old infarction. This was also the case when infarction pain and abnormal Q waves in the ECG were considered.

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CLINICAL EXPERIENCE WITH DETERMINATION OF FIBRINOGEN DEGRADATION PRODUCTS

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Abstract. Sera from 3 075 patients with various diseases and urine from 75 of them have been examined for fibrinogen degradation products (FDP) by the quantitative immunochemical method of Nilého. Such products were found in serum in cancer (61%), chronic renal disease (37), acute renal disease (78%), shock due to sepsis (100%) or postoperative bleedings (100%), acute stage of thromboembolism (72%) and in the acute stage of collagen diseases (38%). In patients with toxemia or hepatosis or other complications of pregnancy FDP were found in 61% and 81% respectively. FDP were found in the urine in almost the same frequency as in the serum in chronic renal disease (urine 39%, serum 37%) and in acute renal disease (urine 57%, serum 78%). FDP in serum were rarely found in liver diseases (<25%) and in the chronic stage of thromboembolism (3%). Uncomplicated hemorrhage did not seem to result in any FDP. These findings appear to warrant the conclusion that repeated demonstration of FDP by our method indicates the presence of some disease requiring further investigation, and secondly that the method might prove a useful diagnostic screening tool.

The last five years have witnessed a remarkable and sustained surge of interest in the occurrence, chemistry and properties of fibrinogen degradation products (FDP) in various conditions (17 29 33 46). The degradation of fibrinogen and fibrin by plasmin occurs over several intermediate stages. The degradation results initially in the formation of high molecular weight split products designated by Marder (29) as *X* (mol. wt. 270 000) and *Y* (mol. wt. 165 000). Complete breakdown of the fibrinogen results in formation of several fragments. Two of them, the D (mol. wt. 80 000) and E (mol. wt. 50 000) components have antigenic determinants in common with fibrinogen (29 33).

Semiquantitative immunological methods have been used for determining FDP in the serum (12, 37). More sensitive than these ... are the immunological procedures based on the Boyden technique. Of these the one most widely used is the hemagglutination inhibition immunoassay by Merskey et al (31). The method is, however, fairly insensitive to D and E products when either is the predominant or sole antigen present (15 16, 19 31 43). In addition precise quantitation by this method may be difficult. Nilého's (33) immunochemical method however has proved very useful for quantitative determination of FDP.

In normal persons FDP have been found in an amount of less than 10 μg ml (10 31 51). Degradation products in the serum have been determined in small series of patients with different conditions. FDP have been demonstrated in varying quantities, mainly in the presence of fibrinolytic conditions (24 34 35), therapeutic fibrinolysis (37), obstetric complications (3), cancer (31) and renal diseases (6 22, 43 47). Recently FDP have also been demonstrated in urine from patients with renal diseases in an active stage (6 22, 43) and after renal transplantation (1 6 7 44).

We have determined the FDP in the serum and urine in a large clinical series by Nilého's immunochemical method (33) in order to find out the possibly diagnostic significance of their presence.

MATERIAL AND METHODS

FDP in the serum and the urine were determined in 3 075 patients referred to our laboratory for investigation. The material is summarized in Table I.

Determination of FDP in serum and urine. Blood samples were collected with E-aminocaproic acid (EACA) to prevent fibrinolysis in vitro. No EACA was added to the urine (stored at +4°C) (4). Degradation products were determined by the immunochemical method devised

Table I. *Diagnoses in patients examined for FDP*

Diagnoses	No. of pats.
Cancer	162
Blood disorders	[illegible]39
Dysproteinemia	190
Arteritis	25
Liver diseases	276
Thrombosis	436
Myocardial infarction	83
Infectious diseases	1[illegible]4
Renal disease	139
Hematuria	87
Pregnancy	1 054
Bleedings	224
Postoperative patients	36
Total	3 075

Table II. *FDP in sera from patients with cancer*

Cancer	FDP		
	No. of pats.	Pos. ( )	Range (mg/100 ml)
Miscellaneous	135	60	0.5–17
Ovarian	14	93	0.5–7
Ut. cervix	13	38	0.5–7
Total	*162*	*61*	

Table III. *FDP in sera from patients with different blood disorders*

Blood diseases	FDP		
	No. of pats.	Pos. ( )	Range (mg/100 ml)
Acute leukemia	43	42	0.5–5
Chronic leukemia	45	22	0.5–3
Thrombocytopenia	77	10	0.5–
Thrombocytosis	14	36	0.5–2
Polycythemia	27	1	
Hemolytic anemia	14	29	1 3
Aplastic anemia	10	20	0.5–3
Agranulocytosis	9	33	1 3

Nilehn (33). In this method an antiserum against the D-fraction of the degradation products is applied to agarose gel. On high voltage electrophoresis serum (diluted 1:1) and urine (unconcentrated, diluted 1:1) migrates into the gel. If FDP are present, they will produce precipitation peaks. The height of such peaks is measured and related to a standard of high molecular weight substances (HMWS). In the presence of EACA this method will not demonstrate any FDP in the serum or the urine of healthy controls (200 cases assayed).

RESULTS

Cancer (Table II). FDP were determined in 162 cases of different sorts of malignant tumours in different stages. FDP were found in the serum of 99 (61 %) and in 13 of 14 cases of ovarian carcinoma.

Blood disorders (Table III). FDP were found in the serum from 4[illegible] % of patients with acute leukemia and in 22 % of those with chronic leukemia.

As regards other blood diseases, FDP were rather common in patients with thrombocytosis.

Dysproteinemia (Table IV). Only 10 [illegible] of the patients with *myeloma* had FDP in the serum. Of the patients with *collagenosis* (rheumatoid arthritis, LED, polyarteritis nodosa) 38 [illegible] had FDP in the serum. In most of these positive patients the disease was in an active phase. Of 29 patients with macroglobulinemia, 7 ([illegible]4 [illegible]) had FDP in the serum.

Arteritis (Table IV). In none of 25 patients with various sorts of arteritis could FDP be demonstrated.

Thrombosis (Table V). FDP were found in

Table IV. *FDP in sera from patients with dysproteinemia, different types of arteritis and liver diseases*

	FDP		
	No. of pats.	Pos. ( )	Range (mg/100 ml)
Myelomatosis	119	10	1 3
Collagen diseases	42	38	1 5
Macroglobulinemia	29	4	1–4
Arteritis	[illegible]5	0	0
Liver diseases			
Cirrhosis	183	[illegible]5	0.5–3
Hepatitis	33	6	0.5–
Unclassified icterus	60	20	0.5–3

Table V. *FDP in sera from patients with thrombosis and myocardial infarction*

	FDP's		
	No. of pats.	Pos. (%)	Range (mg/100 ml)
Thrombosis			
Acute	73	72	1 16
Non-acute	361	3	1 3
Myocardial infarction	83	8	0.5–3

Table VI. *FDP in sera and urine from patients ith renal diseases and hematuria*

	FDP/s			FDP/u		
	No. of pats.	Pos. (%)	Range (mg/100 ml)	N of pats.	Pos. ( )	Range (mg 100 ml)
Renal disease						
Chronic uremia	76	37	0.5–6	31	39	1–11
Acute uremia	18	78	1–8	14	57	35
Hemodialyzed patients	45	44	1–4	19	32	1 11
Hematuria	87	13	0.5–3	11	9	

7'% of 75 patients with acute thrombosis. 361 patients were examined more than three months after the acute attack of thrombosis and in only 3% of these were FDP demonstrable.

Myocardial infarction (Table V) FDP were demonstrable in only 8% of 83 patients with myocardial infarction. Of the patients with FDP one had acute pulmonary embolism, one was found post mortem to ha e widespread ence phalomalacia and one had extensive vascular oc clusions in the lower limbs on the basis of ar teriosclerosis and had had repeated episodes of thrombosis.

Renal diseases (Table VI) Of 76 patients with *chronic uremia* the serum was examined for FDP in 76 and the urine in 31 Such products were demonstrated in the serum in 37% and in the urine in 39%. The occurrence of FDP varied with the severity of the uremia. The patients with FDP were regarded as being in a clinically ac tive stage. The cases without FDP showed no signs of active disease. Of 18 patients with *acute uremia* FDP were demonstrated in the serum in 78% and of 14 patients in the urine in 57%. The concentration of FDP was usually higher in the urine than in the serum. Of *patients being treated with hemodialysis* 44% were found to have FDP in the serum and 32% in the urine. The occurrence of FDP varied with the severity and the activity of the uremia.

Hematuria (T ble VI). FDP in the serum were demonstrable in only 13% of the patients, i.e. in 11 of 87 patients studied, and in the urine in only 1 out of 11 cases studied.

Infectious diseases (Table VII). Serum FDP were found in 23% of the patients with *bronchopneumonia* and in 5 (71%) of 7 with *influenza pneumonia*. In all 13 patients with *sepsis with*

Table VII *FDP in sera from patient h sepsis pneumonia and miscellaneou fection*

	FDP		
	No f pat	Pos (	Ra ge mg 100 m
Sepsis with shock	13	100	1 100
Sepsis without shock	10	70	0 5–8
Bronchopneumonia	39	2.	0 –4
Influenza pneumonia	7	71	4–9
Miscellaneous infections	55	56	5–4

Table VIII. *FDP in sera from 1 054 pregnant ome*

	FDP		
Complications	No. of cases	Pos ( )	Range (mg 100 ml)
None	806	19	0 5–1
Hepatosis	53	81	0 5–3
Toxicosis	195	68	0.5–3

Table IX. *FDP in sera from patients during heavy bleeding*

	FDP/s		
	No. of cases	Pos. (%)	Range (mg/100 ml)
Hemophilia A and B W thrombasthenia	53	3	0 5–2
Menorrhagia	31	16	0.5–3
Melena, hematemesis, nose bleeding etc.	64	27	0 5–2
Postoperative bleedings			
With shock and/or I. coag.	20	100	1–44
Without shock and/or I. coag.	48	17	1 5

shock and/or signs of intravascular coagulation (reduced P & P factor V fibrinogen and decreased platelet count) and in 7 of 10 with *sepsis without shock* or signs of intravascular coagulation, FDP were demonstrated. Of patients with *miscellaneous infections* (mononucleosis, obscure diseases with fever cholecystitis etc.) FDP in the serum were found in 56%

Liver diseases (Table IV) FDP were found in the serum in only 25% of the patients with *liver cirrhosis*

Pregnancy (Table VIII). Of 806 apparently healthy pregnant women examined in the last trimester 19% were found to have FDP in the serum. Most of them had only traces of FDP Among the women with signs of toxicosis and hepatosis (itching and increased SGOT and SGPT) 68% and 81% respectively had FDP in serum.

Heavy bleedings of various etiology (Table IX). *Bleeding diseases.* Fifty-three patients with hemophilia A or B von Willebrand's disease or various types of platelet defects were examined during heavy bleeding periods. Of these, FDP were found in the blood in only 2 (3%). Of 31 patients with *profuse menstrual bleedings* examined, only 5 (16%) had FDP in the serum. Of 64 patients with *melena, hematemesis epistaxis* etc., 27% had FDP in serum. Among 68 patients with *postoperative bleedings* 20 with and 48 without signs of shock and/or intravascular coagulation, FDP were regularly found in the serum in the former group against only 8 (17%) in the latter

Postoperative patients. Thirty-six patients without complications were examined for FDP in the serum up to six days after the operation (cholecystectomy operation for hernia, gastric resection, explorative laparotomy mammary tumour etc.). Small amounts of FDP were found on at least one occasion in 25 of these patients.

DISCUSSION

FDP may occur in the serum and/or urine as a result of intravascular or extravascular breakdown of fibrinogen or fibrin. Fibrin may be primarily deposited in vessels or tissues and be broken down by local or generalised fibrinolysis. The cause of the deposition of fibrin may vary from one disease to another.

FDP are very common in malignant diseases. They were found in the sera from 61% of such patients. It is noteworthy that 13 of 14 with ovarian cancer had FDP in the serum. In several of our patients FDP appeared in the serum before the diagnosis had been recognised in any other way The patients with malignant diseases and FDP had, with but few exceptions, not shown any other signs of fibrinolysis or intravascular coagulation. The fibrinogen and the content of inhibitors of fibrinolysis (inhibitors of the plasminogen activation) were often increased. It is possible that the FDP in these cases derive from extravascular breakdown of fibrin by malignant cells. Several workers (8, 40, 41 45 50) have shown that certain tumours contain fibrinolytic substances.

Of particular relevant interest are renal diseases, in which fibrin deposits have often been found in the kidneys (25 27 48), and such deposits have been thought to be of etiological significance (21 47) in the development of renal diseases. Kincaid-Smith (?0) has therefore recommended anticoagulant treatment of patients with renal diseases at an early stage in order to prevent the development of irreversible renal changes. FDP were found in the serum in 37% of our cases of chronic uremia. In 39% of these, FDP were also demonstrable in the urine, where they were found to be of high molecular weight type (4) The occurrence of FDP also proved to vary with the severity and activity of the uremia. FDP were demonstrable in serum from 78% of the patients with acute uremia. In these the amount of FDP also varied closely with the course of the disease. In 57% of the cases of acute uremia we found HMWS in the urine. All of the patients were subjected to a complete coagulation and fibrinolysis investigation (22). This examination showed no signs of disseminated intravascular coagulation. Our findings, however supported Gilchrist and Liebermann's (15) and Merskey's (30) theory that in renal diseases there may be local coagulation with fibrin deposits in the kidneys. These deposits may possibly afterwards be dissolved to a varying extent by activators of fibrinolysis in the renal tissue (39). This might result in the formation of FDP which are excreted in the urine and to a less extent released to the bloodstream. FDP in the serum may also derive from extrarenal sources, e.g. deposits of fibrin in the pericardium, pleura etc. In patients being

treated with hemodialysis it is possible that the FDP derive also from other extrarenal sources, e.g. deposits of fibrin in the shunts. Together with Dr Bouma (4) we found only HMWS in the urine from patients with renal diseases. During thrombolytic treatment with streptokinase we have also observed FDP in the urine. These FDP have, however proved to consist only of D and E products (4). In animal experiments Rayner et al. (43) have shown that D and E products are readily cleared in the urine, while HMWS are minimally if at all, excreted by the kidneys. Only exceptionally (1 of 8 cases) have we demonstrated FDP in the urine from patients with hematuria. Our experience and reports by others (6, 18, 43 47 49) may suggest the following conclusions. The occurrence of FDP in renal diseases suggests that the disease is in an active stage. The demonstration of HMWS in the urine is a sign of renal disease with deposits of fibrin in the kidney In patients with renal disease, examination of the serum and of the urine for FDP may be of value for deciding whether anticoagulant treatment is indicated or not and for assessing the effect of therapy and the course of the disease.

Our investigations have shown that FDP in the serum seem to be more common in patients with acute leukemia (42 %) than in those with chronic leukemia (22 %). Several of the patients were also found to have decreased fibrinogen and plasminogen and periodically increased fibrinolytic activity in the blood, indicating the presence of a fibrinolytic component. Several workers (5 9 38 and others) have previously shown that episodes of fibrinolysis may occur in leukemia.

Of patients with acute thromboembolism we found FDP in the serum in 72 % suggesting active thrombolysis of the fresh thrombus. Among patients with thrombotic diseases in the inactive phase and those with myocardial infarction FDP were found only occasionally (3 % and 8 % respectively). Six of the seven patients with myocardial infarction and with FDP in the serum had coexisting diseases which might be capable of giving rise to FDP In this connection it should, perhaps, be pointed out that most (77 %) of the patients with bronchopneumonia were not found to have FDP in serum. It is, however interesting to note that most of the patients with influenza type A pneumonia had FDP in the serum.

Of the patients with myelomatosis, FDP were found in 10 % in serum. Nilehn and Nilsson (36) have previously shown that the fibrinolytic activity in the blood was increased in 53 % of all cases of myeloma. In such cases FDP may thus derive from fibrinolytic degradation of fibrinogen.

Of the patients with different collagen diseases 38 % had FDP in the serum. Most of them were found to be in an active phase of the disease. It has been previously shown by various investigators (21 27 47) that in these diseases, especially LED there are subendothelial changes in Bowman's capsule. All patients with other types of arteritis (e.g. Takayasu's disease) had no FDP in the serum.

As known (2, 11 13) the fibrinolytic activity in the blood may be increased in liver cirrhosis. Of our patients 35 % were found to have FDP in the serum.

In recent years the role played by disseminated intravascular coagulation in the pathogenesis of, inter alia, shock of various etiology has been widely discussed (42). Microthrombi in this condition are thought to be formed in a wide variety of organs, especially in the kidneys (23 26). In our material there were 13 patients with shock and/or simultaneous signs of intravascular coagulation. All of them were found to have FDP in the serum, which may probably derive from small microthrombi, dissolved by the activators of fibrinolysis in the vessel walls.

Of patients with different infections (cholecystitis, fever mononucleosis etc.) 56 % had FDP in the serum. Secondary dissolution of fibrin deposits owing to inflammatory processes may in these cases be a possible explanation of the occurrence of FDP in the serum.

To find out whether FDP occurred in the serum in association with heavy bleedings, 53 patients with bleeding disorders were examined. It was interesting to note that all of these patients except two had no FDP in the serum. Also in patients with profuse menstrual bleedings only a few had FDP in the serum. Patients with different types of other bleedings (melena, hematemeses, nose bleedings etc.) had FDP in 27 % In these, however there might have been some other coexisting disease which also caused bleeding and the occurrence of FDP Thus it does not seem probable that FDP will appear in association with heavy bleeding only On the other hand, FDP



were regularly found in patients who had been in a state of shock owing to postoperative bleeding. The cause of FDP in these cases may be the same as that after shock with sepsis (42)

Our findings in the investigation of pregnant women indicated that FDP are fairly common in the presence of complications of pregnancy such as toxicosis and hepatosis. Several investigators have described deposits of fibrin in minute vessels of the liver and in the kidney in toxicosis (28 32), and FDP may thus derive from such deposits.

To find out whether healing after normal, uncomplicated operations is accompanied by FDP in the serum, 36 patients were examined daily on the first six days after operation, and 25 of them were found to have small amounts of FDP in the serum on at least one occasion.

We thus feel that constant demonstration of FDP by the method used by us is a sign of a pathological condition requiring further investigation. Routine examination for FDP in certain cases may be of value as a screening method. In patients with suspected malignant diseases, renal diseases, postoperative complications and sepsis, and during pregnancy determination of FDP already seems to be able to give valuable information. Determinations of the FDP are also useful following the course of a disease and for following the effect of treatment such as anticoagulant therapy

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THE VASOPRESSIN TEST AS AN AID IN THE EVALUATION OF HYPOTHALAMO-PITUITARY ADRENAL FUNCTION

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Abstract. The effect of lysine vasopressin (LVP) on plasma 11-hydroxycorticosteroids (11-OHCS), when administered intramuscularly at 10 p.m., and at one and two hours later has been studied in series of 164 individuals, 43 of whom served as controls and the others represented groups of patients with hypopituitarism, glucocorticoid-treated patients and other metabolically interesting patients. There was a significantly smaller average rise of 11-OHCS in the hypopituitarism group and in the corticoid-treated groups than in the control group. Oestrogen-treated patients responded normally to LVP. The side-effects caused by the administration of LVP were slight and well tolerated in all patients. In a number of patients the adrenal function as studied with the aid of the 5 hours intravenous adreno-corticotropic hormone (ACTH) test after the LVP test. The effect of LVP on the eosinophil cells in the blood was smaller than that caused by ACTH in the test. The LVP test can be used as convenient screening test of the hypothalamic-adrenal system.

Intravenous or intramuscular administration of pitressin and of synthetic lysine-8-vasopressin (LVP) results in elevated plasma corticoid levels in man (6, 13 15). The effect is probably exerted by increasing the secretion of adreno-corticotropic hormone (5 7 20 25) either directly on the pituitary or as a substance with an effect resembling corticotropin-releasing factor or acting on the median eminence of the hypothalamus, by releasing natural CRF as shown in experiments on rats (8). A direct effect of LVP on the adrenal cortex has also been claimed on the basis of rat and dog experiments (9 17). Hence the diagnostic value of the LVP test is not fully established.

It has been suggested that the LVP-induced increase of plasma corticoids could be employed in the assessment of pituitary adrenocortical function (4 6, 22, 23). The series of patients studied so far are small. In a comparison of different modes of administration for clinical purposes, the intramuscular injection of 10 pressor units was shown to be most suitable (4).

The usefulness of the intramuscular injection of 10 units of synthetic LPV was here studied in a clinical series of 164 patients including controls, patients treated with corticosteroid, with pituitary disturbances, and patients suffering from various endocrine disorders.

MATERIAL AND METHODS

The vasopressin preparation used (Postacton® Ferring, LVP) was injected intramuscularly in dose of 0.5 ml, corresponding to 10 units Postacton, at 10 p.m., and blood for determination of the basal 11-OHCS was drawn at 8 a.m. (±15 min) and at one, two and four hours after the injection of LVP. In two obese patients and in one acromegalic patient using an oestrogen preparation, LVP was given as an intravenous infusion. In these cases the dose, infused during an hour, was half of that used in the intramuscular injections. In 8 control cases physiological saline (4 cases) and distilled water (4 cases) were injected intramuscularly in the same volume as LVP.

For the purpose of checking the function of the adrenal cortex in the subjects tested, 20–25 IU Corticotropin Organon in 100 ml saline was infused intravenously during 5 hours and the plasma 11-OHCS was determined beforehand and at 2 / and 5 hours, starting at the same hour of the day as the LVP was injected earlier.

The plasma 11-OHCS content is determined by technique somewhat modified according to Spencer-Peet (18), using Turner fluorometer model 110. Two ml serum is used for the procedure and the concentration of 11-OHCS is calculated by the formula:

(reading of the sample at 10 min 2)
− (reading of the sample at 20 min)

(reading of standard at 10 min 2)
− (reading of standard at 20 min)

conc. of standard (µg/100 ml).

Table I *Results of LVP tests in various patient groups Average plasma 11 OHCS values in μg/100 ml ± S.D before and after LVP*

	No. of pats.	Before LVP	1 h after LVP	2 h after LVP	4 h after LVP	Difference bet een starting value and 1-h alue
Controls	53	15.97±4.48	27 62±6.62	19.25±6 02	10.89±5 6 (– 1)	11.65±6.28
Hypopituitarism No substitution	10	15.12±6.26	20.65±5.37			5.53±3.95
Hypopituitarism with corticosteroid substitution	14	7.91±7.22	11 44±9.58	7 60±5 79		3.53±4 49
Corticosteroid-treated ≥ 7.5 mg prednisolone	15	6 44±7 11	7.20±7.34	5 02±4.77 (n – 14)	4.48±3.87 (8)	0.76±2.68
Corticosteroid-treated 2.5–5 mg prednisolone	11	15.32±4.55	20.85±9.01			5.53±6.95
Secondary oligo-menorrhoea	19	16.60±4.34	28.70±6.33			12.10±6.61
Secondary hypo-gonadism	8	15.59±6.01	24 45±5.76			8.86±9.50
Obesity	19	16.78±7 37	26.31±10.41			9.53±11 43

The standard reading is corrected according to a small but measurable increase in fluorescent intensity

The eosinophil leucocytes were counted in a wet chamber after staining with phloxine in propylene-glycol solution. The verage values of the counts in two chambers were considered.

Table II. *Results of LVP tests in individual cases and in tests repeated with intravenous injection*

Diagnosis	Serum 11-OHCS Before	1 h after LVP	Difference between starting value and 1-h value
Turner syndrome	13 7	59.5	45.8
Turner' syndrome	11 4	27.6	16.2
Turner's syndrome	13.5	33 7	20.2
Obesity (i.m.)	16.0	16.2	0.2
Obesity ( )	10 3	26 6	16.3
Obesity (i.m.)	11.6	14.9	3.3
Obesity (i.)	11.6	25 0	13.4
Oestrogen-gestagen treated	26.0	41.0	15.0
Oestrogen-gestagen *treated*	34 8	53.0	18.2
Oestrogen-gestagen treated	37.3	46.8	9.5
Oestrogen-gestagen treated	8 1	22.7	14.8
Oestrogen-gestagen treated	27.8	60.3	32.5
Oestrogen-gestagen treated	26.6	39.0	12.4
Acromegaly + oestrogen (i. .)	32.9	30 0	−2.9

As controls served 53 patients without clinical evidence of endocrine, hepatic or renal dysfunction, and without severe psychic disturbances, who were not receiving hormone treatment.

Twenty-five patients had received *corticoid treatment* prior to the test. They were divided into two groups: in 15 patients previous dose of corticosteroids corresponding to 7.5–15 mg prednisolone daily during an verage period of 6 / (range 1/12 10) years had been used. In 11 other patients the previous corticoid therapy consisted of 2.5–5 mg prednisolone or some corresponding preparation during an average period of 4 / (1/12–10) years. No cortisone preparation was administered later *than the morning of the day before the test.*

I 10 patients *pituitary disease* was observed, but the patients had not had any hormone substitution. Seven of these patients had pituitary adenomas, two had been operated on for pituitary adenoma and one was an untreated pituitary dwarf.

In 10 patients *h pophysectomy* had been done prior to the test because of mammary cancer (5 cases), chromophobic adenoma (1 case), progressive exophthalmos (1 case), acromegaly (2 cases) and craniopharyngeoma (1 case). The test was done twice in one f the patients suffering from mammary cancer and in one with chromophobic adenoma. T patients suffering from pituitary dwarfism were also included in this group. All these patients received substitution therapy with corticoid hormone at the time of the test.

In 19 patients *secondary oligo- or amenorrhoea* ithout other endocrine disease was obvious *from clinical* and anamnestic data.

Another group consisted of 19 *obese* patients (3 f them male). Their eight exceeded the normal weight of individuals of the same height, according to standard figures by 15% or more. I two of these cases LVP was

Table III. The effect of intravenous infusion during 5 hours of 20–25 units ACTH on plasma 11 OHCS (μg/100 ml) ± S.D. in different groups

	No. of pats.	Before ACTH	At 5 h during infusion	p-value for 5-h value in comparison to the value in the control group
Controls	53	14.91 ± 5.42	50.3 ± 12.74	
Hypopituitarism No substitution	10	13.61 ± 3.22	46.6 ± 8.32	—
Hypopituitarism with corticosteroid substitution	14	4.96 ± 3.10	18.26 ± 10.16	0.001
Corticosteroid-treated ≥ 7.5 mg prednisolone	15	6.63 ± 6.23	15.91 ± 13.32	0.01
Corticosteroid-treated with 2.5–5 mg prednisolone	11	15.27 ± 1.95	36.93 ± 9.96	0.01
Secondary oligomenorrhoea	19	14.28 ± 3.55	45.71 ± 11.07	—
Secondary hypogonadism	8	10.20 ± 3.78	41.08 ± 7.13	0.05
Obesity	19	14.22 ± 4.11	49.48 ± 14.4	—

administered intravenously because of an insufficient response to the intramuscular injection (Table III).

In 8 cases *male hypogonadotropic hypogonadism* was obvious either because of several years delay in puberty in relation to chronological age of 17 years or more (5 cases) or as clinically manifest hypogonadotropism with low excretion of gonadotropic hormones in urine and testicular histology typical of hypogonadotropic hypogonadism (3 cases).

The test was also done in 6 *patients using oestrogen hormones* as contraceptives or in corresponding dosage and in one typical case of *acromegaly on oestrogen* therapy. Three cases of *Turner's syndrome* with typical sex chromosome karyotypes were studied as well.

RESULTS

Control injection without LVP. The results of 8 injections of saline or distilled water (4 of each) intramuscularly in the same volume as LVP and at the same time of day gave the following results: before the injection 11-OHCS was 14.5 (range 6–24) μg/100 ml, 1 hour after 12.5 (6–20) and 2 hours after 11.1 (7–21.7) μg/100 ml. Apparently these injections had no effect on the plasma 11-OHCS level.

The results of the LVP tests. The effects of intramuscular injections of LVP on the plasma 11-OHCS content in the control group and in the groups of endocrinological interest are given graphically in Fig. 1. Table I shows the corresponding average values of 11-OHCS and the standard deviation (S.D.) before the injection of LVP, after 1 hour, after 2 hours, and in a small number of cases after 4 hours, as well as the difference between the 1-hour and initial values of 11-OHCS. Using the *t*-test for statistical evaluation, the results of the LVP tests reveal highly significant differences between the average increase of 11-OHCS after LVP in the control group compared to the corresponding increase in the groups of patients with pituitary deficiency with or without steroid substitution and in the

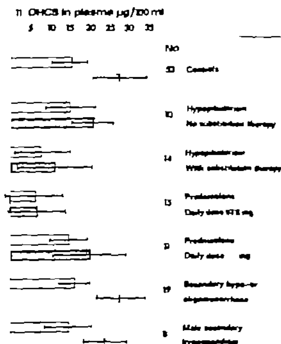


Fig. 1. The mean values with S.D. of plasma 11-OHCS before and 1 h after intramuscular administration of LVP in control group and in various groups of patients.

groups treated with small and medium-sized doses of corticosteroids ($p = 0.001$ in all groups). The differences between the average increase of plasma 11-OHCS in the control group compared to the corresponding increase in the groups of patients with secondary oligomenorrhoea, secondary male hypogonadism and obesity were not regarded as significant (cf Fig. 1), although the p-value for the difference between the increments in the control group and in these groups (0.01 0.02, 0.01 respectively) indicated the possibility of disturbance somewhere in the hypothalamus-pituitary adrenal system in some cases in these relatively small heterogenous groups.

In the obese group LVP was twice administered intravenously in a dose of 5 units Postacton® because a previous intramuscular injection did not cause a rise of 11-OHCS. The results of these tests are shown in Table II. There was a significant rise of 11-OHCS in the test made with intravenous administration of LVP

Table II includes the results of LVP tests in 6 healthy individuals who were using contraceptives (5 subjects) or an oestrogen preparation (1 subject) in corresponding doses. Although the initial level of 11-OHCS was mostly higher than in the control group, there was a clear rise of 11-OHCS in all the subjects taking oestrogens. The average rise was even higher than in the control group (17.1 μg against 11.96 μg) Further Table II shows the results of the LVP test in one case of acromegaly on oestrogen therapy The 11-OHCS content in the plasma did not rise in this case, although LVP was administered intravenously in a dose of 5 units.

Three cases of Turner's syndrome are also included in Table II. In all cases there was a clear increase of 11-OHCS.

The results of intravenous ACTH infusions. The effect on the plasma 11-OHCS content of ACTH administered intravenously during 5 hours was studied in a number of patients in the different groups (Table III) with the purpose of gaining a clearer picture of the functional state of the adrenal cortex. This is of importance for evaluating the results of the LVP test. The table also includes a comparison with the t-test between the values after 5 hours in the control group and the corresponding values in the other groups. The increase of 11-OHCS in the group of patients with pituitary deficiency did not significantly differ from the result in the control group ($p > 0.1$), whereas the difference between the control group and the corticosteroid-treated groups was significant ($p = 0.01$).

The effect of LVP and ACTH on the eosinophil count In a number of cases in the control group the eosinophil cells were counted in the peripheral blood before and after administration of LVP and the lowest value within 4 hours after the LVP injection was used for comparison. The eosinophils were also determined before the intravenous ACTH injections and at 5 hours during the infusion. The lowest value of the eosinophils after injection of LVP was 49.72 ± 6.42 % (standard error of deviation) of the initial value and during the intravenous ACTH infusion 13.59 ± 14.25 % at 5 hours.

The side-effects caused by the LVP consisted of pallor gastric distress, often with a tendency to diarrhoea and sometimes headache or general discomfort. These effects were never of a serious nature or really troublesome. The patients were warned before the test of the possibility of side-effects. The test was not done in cases with known coronary sclerosis.

DISCUSSION

The theoretical foundation for the use of the response of plasma 11-OHCS to vasopressin administration is, as mentioned in the introduction, not quite settled. In the rat, vasopressin causes release of corticotropin, and probably acts at a hypophysial site (25). The rise of plasma steroids in man caused by intramuscular vasopressin administration is, according to some investigators (6), blocked by premedication with either morphine or dexamethasone (5), but others deny the inhibiting effect of prednisolone (3). ACTH itself diminishes the steroid response to vasopressin (21).

It has been recommended that the LVP test should be performed in the afternoon because of the supposedly increased sensitivity of plasma corticoid production at that time. Others have been unable to detect any difference between the response to LVP in the forenoon and in the afternoon (4 16) The maximal rise of 11-OHCS after the injection of LVP apparently occurs at 30–60 min (4 22). False pathological tests have sometimes been reported without any apparent explanation (?6).

Table IV *The increment the maximal level and the starting value of plasma 11 OHCS in connect on h LVP tests in different groups*

	No. of tests	11-OHCS increments after LVP < 5 µg/100 ml (no. of pats.)	Maximal 11-OHCS after LVP < 16 µg/100 ml (no. of pats.)	Basal level of 11-OHCS < 5 µg 100 ml (no. of pats)
Controls	53	6	1	0
Hypopituitarism No substitution	10	5	3	1
Hypopituitarism with corticosteroid substitution	14	10	10	6
Corticosteroid-treated > 7.5 mg prednisolone	14	14	12	11
Corticosteroid-treated < 5 mg prednisolone	11	6	7	0
Secondary oligo-menorrhoea	19	3	1	0
Male secondary hypogonadism	8	3	1	0
Oestrogen-gestagen treated	6	0	0	0

In our 53 control cases there was a significant average rise of 11-OHCS an hour after intramuscular administration of 10 units LVP whereas there was no response to an intramuscular injec tion of the same amount of saline or distilled water. In two of the controls there was no rise of the plasma 11-OHCS.

In two obese patients in this series no rise of 11-OHCS was observed. However when the test was repeated on another day and LVP was administered intravenously a clear response of 11 OHCS was seen. Thus apparently deficient re sorption of LVP may be one cause of an other wise inexplicable negative response. A deficient 11-OHCS response to LVP in patients with Cush ing s disease due to adrenal cortical adenomas or non-endocrine tumours has been reported (2, 23), but not in adrenal hyperplasia (2).

The side-effects of LVP were never serious and mostly very mild. It seems to be important t prepare the patient for possible distressing symptoms in connection with the test. The test should not be carried out on patients suspected of coronary insufficiency Airway obstruction was once reported after LVP (4).

A significantly reduced verage response of 11-OHCS to LVP compared to the response in the control groups was seen in the groups of patients with pituitary failure not pretreated with normal response of 11-OHCS to ACTH and pretreated with hormones, and in the groups of corticosteroid-treated patients. The reduction of the response to LVP was inversely related to the sterod dosage used.

When considering the effect caused by LVP on the eosinophil cell count it seemed probable that LVP in the dosage used was a weaker stim ulant of the 11-OHCS production than ACTH in the intravenous test.

Brostoff et al. (4) used as criteria of a normal VPT response a basal plasma level of 11-OHCS exceeding 5 µg/100 ml, a minimal level reached during the test of 16 µg/100 ml, and an increment above the basal value of at least 5 µg/100 ml. As the average basal value of plasma 11-OHCS in our series, 15.97 µg/100 ml, came close to the corresponding value of Brostoff et al., 13.9 µg/100 ml, and the mean increment was 11.96 in our series, against 12.1 in theirs, we used the same criteria for comparison. The results are seen in Table IV They correspond well to those reported by Brostoff et al.

With regard to the report that the response to the metyrapone test is less strong in subjects receiving oestrogen (19) it was of interest that in the six patients taking oestrogens or an oestrogen-gestagen combination in doses corresponding to 0.05–0.1 mg ethinyloestradiol the rise of 11-OHCS after LVP was normal, although the basal values were as a rule increased.

We were not able to compare our results with those of other tests of the function of the hypo-

thalamic pituitary system. Such comparisons have hitherto been made in small series of patients only with non-uniform disturbances and do not yet allow a definite evaluation of the place and significance of such tests (metyrapone test (MPT) pyrogen and insulin hypoglycaemia stimulation tests) (3 10, 12, 22). The MPT has been widely used but its results are difficult to interpret when the initial urinary steroid excretion is low It determines only the reaction of the pituitary to a falling hydrocortisone level (14) and is thus only of limited value in predicting the response of the pituitary gland to stressful situations. The response may be abolished in patients with myxedema, liver diseases or pregnancy or who are receiving certain drugs, such as oestrogens, anabolic steroids or chlorpromazine Because of diurnal variation in sensitivity to metyrapone, this drug ought to be given at midnight and at 9 a.m. or a false negative result may be obtained (11 26). MPT requires several days for completion and cannot be performed by the rapid fluorimetric method for determining the plasma 11-OHCS.

Anyway the LVP test has the advantage of being simple, and therefore this test has a place at least as a screening method when studying hypothalamic-pituitary-adrenal function in otherwise healthy subjects.

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HYDROXYPROLINE EXCRETION IN THE URINE AND CALCIUM METABOLISM DURING LONG-TERM TREATMENT OF THYROTOXICOSIS WITH PROPYLTHIOURACIL

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Abstract. Thirty hyperthyroid patients have been studied before and during long-term treatment with propylthiouracil for the purpose of evaluating changes in the excretion of urinary hydroxyproline and relating these to some parameters of calcium metabolism and thyroid function. Urinary hydroxyproline and urinary calcium were found to be significantly raised before treatment. A very rapid fall in urinary calcium was noted within the first two weeks of treatment. BMR and PBI showed a parallel fall to euthyroid values following six weeks of treatment, while there was a slow fall in the mean value of urinary hydroxyproline. After three months the mean value was still elevated and normal values of urinary hydroxyproline were not obtained until six months after the beginning of treatment. A possible causal relation between collagen metabolism and calcium metabolism during antithyroid treatment is discussed. Our results may indicate that many hyperthyroid patients do not reach a metabolic steady state until about three months after the beginning of antithyroid treatment.

The relation between thyroid gland activity and bone metabolism has been recognized for many years (3). Thyrotoxicosis is generally associated with increased excretion of calcium in urine and faeces, and hypercalcaemia occasionally occurs (5). Mobilization of calcium from the bones with increased activity of the osteoclast seems to be directly stimulated by thyroid hormones. However, secondary changes in parathyroid hormone and possibly also in thyrocalcitonin secretion also influence the alterations in mineral metabolism in hyperthyroidism (1, 7).

In recent years it has been shown that urinary hydroxyproline can be used as an index of collagen metabolism (19), and increased excretion of hydroxyproline has been described in patients with hyperthyroidism (6, 8, 9, 20). In hyperthyroidism the increased excretion of hydroxyproline is caused by increased rates of degradation of both soluble and insoluble collagen (10, 11). Studies by Laitinen (15) on the interaction of collagen and calcium metabolism in animals suggest that the primary action of the hormones affecting bone resorption and formation may be mediated by changes in the metabolism of collagen.

The purpose of this investigation has been to study changes in the excretion of urinary hydroxyproline, some parameters of calcium metabolism and thyroid function in a group of hyperthyroid patients before and during treatment with antithyroid drugs.

MATERIAL AND METHODS

The material comprised 30 patients with hyperthyroidism, 28 women aged 23 to 77 years, and two men aged 50 and 42 years. The diagnosis was based on the clinical picture and several thyroid function tests (BMR, PBI, serum thyroxine, T 3 resin uptake, ^{131}I uptake in the thyroid gland).

The patients were kept on a low-gelatine and low calcium diet for 24 hours before collection of the urine and during the following three days. The urine was collected under toluene and stored at 4°C until analyzed. Total urinary hydroxyproline was determined by a modification of the method described by Prockop and Udenfriend (18). The urine was hydrolyzed with equal volume of concentrated hydrochloric acid in an oven at 140°C for three hours. It was found necessary to readjust the pH quite accurately to the range 8.1–8.3. All determinations were done in duplicate and the results given are the mean value of two 24-hour urine specimens. The normal range for adults (control group 16 persons 17–85 years old) in our laboratory is 28.7 ± 11.8 mg/24 h (mean ± 2 S.D.). Protein bound iodine, serum calcium, urinary cal-

Table I. *Results from 30 hyperthyroid patients before and during long-term treatment with propylthiouracil*

Treatment	BMR (%)	PBI (μg/100 ml)	Serum Ca (mg/100 ml)	Serum albumin (g/100 ml)	Serum alkaline phosphatase (KA units)	Urine Ca (mg/24 h)	Urine OH-PRO (mg/24 h)	
Before	145±20	11.6±3.5	10.1±0.6	3.8±0.46	11±5	213±137	71±32	30
2 weeks	128±18	7.0±3.8	9.8±0.5		12±7	123±74	55±23	30
6 weeks	115±21	4.1±2.8	9.6±0.5	4.3±0.57	14±9	113±61	45±21	29
3 months	114±12	3.6±2.1	9.6±0.4		12±6	124±67	42±19	30
6 months	115±15	3.8±2.4	9.4±0.6	4.3±0.45	10±6	110±52	29±11	16
9 months	115±11	4.4±1.9	9.1±0.4		10±5	111±57	33±11	8
12 months	118±16	5.9±1.6	9.6±0.4		9±8	113±58	42±18	8
18 months	116±17	4.8±1.2	9.6±0.6		8±6	146±51	32±11	9
24 months	110±13	5.4±1.0	9.1±0.3		9±5	130±58	35±9	7
Normal range	90–120	3.5–8.0	8.7–10.7	3.8–5.6	3–13	50–150	17–41	

cium, serum alkaline phosphatases and serum albumin were determined as routine analyses in the Central Laboratory Gentofte Hospital.

RESULTS

The results are shown in Table I. The mean values for basic metabolic rate (BMR) and protein-bound iodine (PBI) showed a parallel fall to euthyroid levels in the course of six weeks of therapy The mean value of serum calcium before treatment cannot be regarded as elevated. During treatment, however a significant ($p < 0.05$) fall was noted. During the same period mean serum albumin levels increased, suggesting a greater fall in ionized calcium during the first months of treatment than indicated by the total serum calcium values. Before treatment the mean value of serum alkaline phosphatases was slightly but not significantly elevated. During treatment the mean value showed no significant changes, but there was a tendency to an increase during the first months of treatment, followed by a decrease. The mean value for urinary calcium was significantly elevated before treatment ($p < 0.01$) and a very rapid fall was noted within the first two weeks of treatment. During the following months *no significant changes were observed, but there* was a tendency to a decrease in mean values followed by an increase.

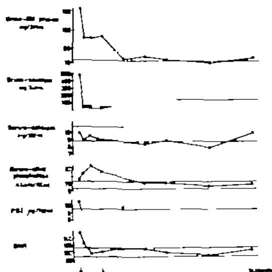


Fig. 1. Urinary hydroxyproline excretion and some parameters of calcium metabolism and thyroid function in hyperthyroid patient before and during long-term treatment with propylthiouracil.

Urinary hydroxyproline was found to be significantly elevated in this group of 30 thyrotoxic patients ($p < 0.01$). Twenty-seven of these patients had urinary hydroxyproline values above the normal range During treatment there was a slow fall in the mean values for urinary hydroxyproline and three months after the beginning of treatment the mean value was still raised. Normal values were not obtained until six months after the beginning of treatment. Thus urinary hydroxyproline *in several cases remained elevated* several months after the normalization of BMR, PBI and urinary calcium, all of which reached euthyroid levels within six weeks of the initiation of antithyroid therapy Fig. 1 shows the same parameters in a typical patient. In this patient the rapid normalization of BMR and PBI and urinary calcium contrasts with the much more prolonged elevations in urinary hydroxyproline excretion and in serum alkaline phosphatases.

DISCUSSION

The values obtained for serum calcium, serum albumin, serum alkaline phosphatases and urinary calcium in our group of thyrotoxic patients before treatment are generally in accordance with those reported previously (3 4 5 16, 17). The exact mechanism of the changes in bone metabolism in hyperthyroidism is not fully understood. Studies using the isotope dilution technique have revealed increased rates of bone formation and resorption and a negative calcium balance (5 14). The increased mobilization of calcium from the bones, probably caused by a direct stimulation of the bone-removing cells by the thyroid hormones (1) leads to a rise in ionized calcium. This rise might cause a decrease in the excretion of parathyroid hormone. Since parathyroid hormone increases the renal resorption of calcium, a secondary hypoparathyroidism may partly explain the hypercalcuria (7 1.) Thyrocalcitonin inhibits bone resorption (2), and reduced excretion of thyrocalcitonin could be compatible with the changes in calcium metabolism in hyperthyroidism.

Reports about variations in calcium metabolism during treatment of thyrotoxicosis are few. Lundbæk (16) and Cook et al. (5) followed small groups of patients during treatment with antithyroid drugs and found a very rapid reduction in urinary calcium within two to three weeks. Our results are in full agreement with these findings.

The mean value for urinary hydroxyproline in a group of thyrotoxic patients was found to be significantly elevated compared with that in euthyroid controls this is in agreement with several previous reports (6, 8 9 11 20). Kivirikko et al. (9) examined urinary hydroxyproline in six hyperthyroid patients before and after about ten days of antithyroid treatment. In three of the patients urinary hydroxyproline was still raised and in the other three patients it had returned to normal levels. Kivirikko et al. (9) suggested that a rapid normalization seemed to occur during drug therapy In contrast to this interpretation we have found that the mean value of urinary hydroxyproline in larger group of thyrotoxic patients receiving antithyroid therapy remained raised for several months after normalization of BMR and PBI.

In a study of the metabolism of collagen and its hormonal control in the rat by Laitinen 5) it has been suggested that the metabolism of collagen in the bones may be of major importance in the hormonal regulation of bone formation and resorption. In rats treated with thyroxine it was found that the increase in urinary hydroxyproline-^{14}C induced by thyroxine preceded the removal of ^{45}Ca from the bones. During treatment with parathyroid extract the changes in hydroxyproline excretion and in calcium metabolism occurred simultaneously To the best of our knowledge there have been no reports of similar studies concerning collagen and calcium metabolism in man during variations in thyroid function. We have found that the mean value of urinary hydroxyproline remained elevated much longer than urinary calcium, BMR and PBI during antithyroid treatment the mean value of alkaline phosphatases in serum also showed an increase lasting for several months. These findings probably partly reflect an increased rate of bone formation following the loss of calcium during the state of hyperthyroidism (4 14 17) However alkaline phosphatases originated from other organs than the bones may also contribute to the elevation of serum alkaline phosphatases found in some patients (Fig. 1).

The interpretation of our results is also rendered difficult by the fact that several hormones other than thyroid hormones affect bone formation and urinary hydroxyproline (13 15) Secondary changes in parathyroid hormone, and possibly also changes in the secretion of thyrocalcitonin, somatotrophin and corticosteroids, may influence the variations found in urinary hydroxyproline and in the parameters of calcium metabolism. The increased urinary hydroxyproline may also reflect increased collagen metabolism in tissues other than bone.

Our results thus do not permit any conclusions to be drawn about a possible causal relation between collagen metabolism, evaluated by urinary hydroxyproline, and the calcium metabolism during antithyroid treatment. The finding of elevated urinary hydroxyproline values three months after the beginning of antithyroid treatment, at time when BMR and PBI had been normal for weeks, may be an indication that even after this period these patients have not reached a metabolic steady state.

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BLOOD GLUCOSE AND PLASMA GLYCEROL RESPONSE TO EPINEPHRINE INFUSION IN NORMAL AND PREDIABETIC SUBJECTS

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Abstract. The effect of epinephrine infusion (at rate of 0.08 to 0.11 μg/kg/min) on blood glucose and plasma glycerol has been investigated in six normal subjects and nine individuals with normal glucose tolerance but decreased and delayed insulin response to glucose (prediabetic subjects). Responsiveness to epinephrine, as measured by the magnitude of elevation in blood glucose and plasma glycerol, was of similar degree in both groups of subjects. It is concluded that the ability to mobilize glycogen and lipid is not altered in prediabetes. The mechanisms responsible for maintaining a normal glucose tolerance in the presence of decreased insulin secretion in prediabetic subjects then do not seem to involve diminished ability to generate cyclic AMP in the liver and fat cells.

Several studies from this laboratory have demonstrated that subjects with prediabetes are characterized by an inability to promptly release adequate amounts of insulin in response to a glucose challenge (6, 7 8). More recent studies (4 10) have indicated that the defective insulin response to glucose in prediabetes may be due to decreased cyclic 3',5'-adenosine monophosphate (cyclic AMP) production by the pancreatic beta cells of prediabetic subjects.

Prediabetics have a defect in insulin release that is similar in magnitude to the defect found in patients with manifest diabetes, but normal glucose tolerance is maintained (7) This suggests that there are mechanisms in prediabetes that compensate for the insulin deficiency (9). Possible mechanisms could be a decreased mobilization of liver glycogen and a reduced rate of lipolysis. Since these two functions are also cyclic-AMP-mediated (1 24) we have speculated that, in prediabetics, the defect in the cyclic AMP system is generalized and the defective insulin release thus counter balanced by decreas[illegible] pr[illegible]d [illegible] tion, resulting in normal [illegible] The present investigat[illegible] to test this hypothes[illegible]

MATERIAL AND [illegible]D

Fifteen healthy non-obe[illegible] venous glucose toleranc[illegible] Nine were considered t[illegible] be p[illegible] criteria mentioned above, i.e. th[illegible] and delayed insulin response [illegible] sulin response being evaluated b[illegible] (3). The remaining subjects had normal i[illegible]

The epinephrine infusions [illegible] performed aro[illegible] m. after 12 15 hours' fasting and with the subjects in recumbent position. 1.5 mg of [illegible]pinephrine (calculated from the corresponding amount of [illegible]pinephrine bitartrat[illegible]) was diluted in 500 ml of physiological NaCl containing ascorbic acid, 2.5 mg/ml, as an antioxidant. Epinephrine [illegible]as administered intravenously at rate of 0.08 to 0.11 μg/kg b wt./min between 30 and 60 min. Glucose [illegible] injected rapidly as dose of 500 mg/kg b. [illegible]t. at 0 min, followed by the infusion of glucose at the rate of 20 mg/kg/min up to 60 min. Blood was draw[illegible] through an indwelling needle into heparinized tubes before and du[illegible]ing the infusions at the time intervals given in Tables I–III. Whole blood for glucose determination [illegible] pipetted off, the tubes then being centrifuged immediately and the plasma being frozen for later determinations of insulin and glycerol.

Blood glucose was measured by [illegible] commercial glucose oxidase method (Kabi Reagens, Stockholm), plasma insulin by [illegible] double antibody radioimmunoassay (17). Plasma glycerol was determined fluorometrically according to Chernick (14).

RESULTS

The levels of blood glucose and insulin and glycerol in plasma during the infusions of epineph-

Table I. *Blood glucose levels during epinephrine (−30 to 0 min) and epinephrine plus glucose (0 to 60 min) infusions in normal (group I) and prediabetic (group II) subjects*

		Dose of epinephrine (μg/kg/min)	Blood glucose (mg/100 ml) −30	−20	−10	0	10	20	30	40	50	60 min
Group I	1	0.08	77	94	119	140	388	426	468	519	573	624
	2	0.09	70	69	76	100	303	394	440	453	502	503
	3	0.09	61	66	77	84	283	314	324	351	382	433
	4	0.10	70	74	90	111	351	390	429	471	496	531
	5	0.10	67	67	78	84	369	417	469	501	533	579
	6	0.11	68	68	87	108	385	428	483	546	605	675
Mean ± S.E.M.			69 ± 2.1	73 ± 4.4	88 ± 6.6	105 ± 8.5	347 ± 17.9	395 ± 17.4	436 ± 23.8	474 ± 28	515 ± 31.6	558 ± 35.4
Group II	1	0.09	63	75	81	89	287	307	358	395	415	446
	2	0.09	78	96	104	109	297	310	362	386	429	447
	3	0.09	71	75	95	103	322	344	367	394	436	463
	4	0.09	68	78	98	103	339	360	397	433	463	477
	5	0.09	69	76	97	109	397	435	538	584	659	701
	6	0.09	82	80	90	112	290	336	398	476	562	576
	7	0.09	68	73	100	109	400	492	578	637	683	764
	8	0.09	72	89	113	150	426	472	526	586	615	644
	9	0.09	65	75	85	91	428	356	647	739	824	893
Mean ± S.E.M.			71 ± 2.0	80 ± 2.6	96 ± 3.2	108 ± 5.9	354 ± 19.6	401 ± 30	463 ± 36.5	515 ± 42.2	563 ± 47.2	601 ± 53.6
Difference I–II p			> 0.5	> 0.5	> 0.5	> 0.5	> 0.5	> 0.5	> 0.5	> 0.5	> 0.5	> 0.5

e II *Plasma insulin levels during epinephrine (−30 to 0 min) and epinephrine plus glucose (0 to 60 min) fusions in normal (group I) and prediabetic (group II) subjects*

		Dose of epinephrine (μg/kg/min)	Plasma insulin (μu/ml) −30	−20	−10	0	10	20	30	40	50	60 min
Group I	1	0.08	24	14	15	17	19	17	22	26	34	37
	2	0.09	26	18	15	17	23	23	26	28	34	35
	3	0.09	23	14	16	21	69	30	30	34	54	90
	4	0.10	27	26	16	19	29	27	33	53	58	66
	5	0.10	17	14	13	15	20	19	22	32	42	47
	6	0.11	25	15	19	22	20	17	24	23	20	63
Mean ± S.E.M.			24 ± 1.5	17 ± 1.9	16 ± 0.8	19 ± 1.1	30 ± 7.9	22 ± 2.2	26 ± 1.8	33 ± 4.4	40 ± 5.8	46 ± 6.2
Group II	1	0.09	25	25	32	34	30	36	40	50	50	54
	2	0.09	19	15	17	15	16	18	22	28	40	45
	3	0.09	20	21	21	18	25	28	28	32	31	41
	4	0.09	25	23	21	20	28	27	29	32	39	39
	5	0.09	19	17	19	17	23	27	44	34	42	52
	6	0.09	23	20	17	16	18	22	19	24	84	47
	7	0.09	1	11	14	16	24	20	20	22	23	25
	8	0.09	20	12	8	8	12	15	17	44	46	52
	9	0.09	13	15	16	16	31	31	36	41	55	69
Mean ± S.E.M.			20 ± 1.3	18 ± 1.6	18 ± 2.2	17 ± 2.3	23 ± 2.2	25 ± 2.2	23 ± 3.3	34 ± 3.1	46 5.8	47 ± 3.9
Difference I–II p			> 0.05	> 0.2	> 0.2	> 0.2	> 0.2	> 0.2	> 0.2	> 0.2	> 0.2	0.2

Table III. *Plasma glycerol levels during epinephrine (−30 to 0 min) and epinephrine plus glucose (0 to 60 min) infusion in normal (group I) and prediabetic (group II) subjects*

		Dose of epinephrine (μg/kg/min)	Plasma glycerol (μM/ml)									
			−30	−20	−10	0	10	20	30	40	50	60 min
Group I	1	0.08	0.110	0.152	0.183	0.191	0.194	0.184	0.177	0.163	0.164	0.148
	2	0.09	0.134	0.143	0.141	0.154	0.120	0.103	0.100	0.090	0.086	0.091
	3	0.09	0.077	0.089	0.117	0.113	0.099	0.075	0.095	0.080	0.093	0.077
	4	0.10	0.073	0.107	0.119	0.143	0.149	0.145	0.137	0.133	0.140	0.117
	5	0.10	0.123	0.136	0.186	0.223	0.242	0.214	0.206	0.221	0.191	0.148
	6	0.11	0.103	0.153	0.178	0.201	0.206	0.217	0.195	0.173	0.148	0.109
Mean ± S.E.M.			0.103 ± 0.010	0.133 ± 0.012	0.154 ± 0.013	0.171 ± 0.017	0.168 ± 0.022	0.156 ± 0.0[illegible]4	0.152 ± 0.020	0.143 ± 0.022	0.137 ± 0.017	0.115 ± 0.012
Group II	1	0.09	0.061	0.081	0.083	0.093	0.079	0.078	0.081	0.096	0.066	0.063
	2	0.09	0.099	0.134	0.148	0.138	0.132	0.124	0.119	0.111	0.108	0.104
	3	0.09	0.068	0.104	0.117	0.114	0.104	0.086	0.084	0.083	0.073	0.073
	4	0.09	0.074	0.091	0.104	0.109	0.100	0.111	0.099	0.094	0.108	0.098
	5	0.09	0.146	0.165	0.153	0.164	0.180	0.160	0.154	0.141	0.140	0.139
	6	0.09	0.160	0.217	0.251	0.256	0.237	0.241	0.180	0.183	0.206	0.[illegible]41
		0.09	0.156	0.217	0.260	0.[illegible]57	0.261	0.233	0.221	0.219	0.192	0.175
	8	0.09	0.094	0.226	0.202	0.181	0.185	0.192	0.202	0.182	0.195	0.211
	9	0.09	0.099	0.110	0.121	0.102	0.176	0.121	0.126	0.114	0.142	0.094
Mean ± S.E.M.			0.097 ± 0.014	0.149 ± 0.019	0.160 ± 0.021	0.157 ± 0.021	0.156 ± 0.021	0.150 ± 0.020	0.141 ± 0.017	0.136 ± 0.016	0.137 ± 0.017	0.133 ± 0.021
Difference I–II p			>0.4	>0.4	>0.4	>0.4	>0.4	>0.4	>0.4	>0.4	>0.4	>0.4

rine alone (−30 to 0 min) and epinephrine plus glucose (0 to 60 min) in the groups of normal subjects and prediabetic individuals are given in Tables I–III.

Blood glucose increased slowly during epinephrine administration from the mean fasting levels of 69 mg/100 ml in normal subjects and 71 mg/100 ml in prediabetics to 105 and 108 mg/100 ml, respectively. During glucose infusion blood glucose reached very high levels, peaking at 60 min 558 mg/100 ml in normals and 601 in prediabetics (Table I).

Plasma insulin decreased significantly in both groups during the infusion of epinephrine alone ($p < 0.01$ in normals, < 0.025 in prediabetics). Insulin response to glucose administration was markedly depressed initially but with increasing blood glucose levels a rise in plasma insulin was noted in both groups (from 19 to 46 μu/ml at 60 min in normals, from 18 to 47 in prediabetics) (Table II).

Plasma glycerol rose sharply in both groups when epinephrine was administered, reaching 0.171 μM/ml in normals and 0.157 in prediabetics at 0 min. When glucose was infused, there occurred a gradual decrease in glycerol concentration in both groups, the levels at 60 min being significantly lower than at 0 min ($p < 0.001$) (Table III).

The parameters studied in this work, i.e. blood glucose, plasma insulin and plasma glycerol showed no statistically significant differences between the groups of normal and prediabetic subjects, this being true both for the fasting state for the duration of epinephrine infusion, and the combined administration of epinephrine and glucose.

DISCUSSION

It seems well established that in prediabetes—the stage that precedes the development of carbohydrate intolerance in the natural history of diabetes—insulin release from the pancreas is defective, i.e. the insulin response to a glucose challenge is delayed and diminished (5–8, 21 23). Although, as a group, severe diabetics show more impairment of insulin release than mild diabetics or prediabetics (7 22) in many prediabetic subjects the impairment of insulin release is of the same magnitude as found in patients with manifest diabetes. This implies, in prediabetes, that certain mechanisms are keeping the glucose tolerance

within normal limits in spite of the insulin deficiency. In other words, insulin seems to be more effective in prediabetic subjects in clearing glucose from the blood. This increased insulin sensitivity can be shown indirectly by calculating the increased biological effect of endogenous insulin during glucose infusions through analogue computation (7), and directly by measuring the increased hypoglycemic effect of intravenously administered insulin in prediabetics as compared to normals and manifest diabetics (11).

The mechanisms by which insulin is rendered more effective in prediabetic individuals remain obscure. We have previously presented data suggesting that a decreased gluconeogenic function of the liver may be one of the mechanisms keeping glucose tolerance normal (9). Two other mechanisms actively participating in the dynamics of glucose tolerance are glycogenolysis in the liver and lipolysis in adipose tissue. Therefore, these functions were examined in prediabetic subjects. For several reasons epinephrine was chosen as the experimental tool to study these two functions: it is a potent glycogenolytic (18) and lipolytic hormone (15, 16) in man; it suppresses insulin release from the pancreas (20), thus eliminating insulin's antilipolytic and antiglycogenolytic activity.

Epinephrine exerts its glycogenolytic and lipolytic functions by activating adenyl cyclase, thus increasing the concentration of cyclic AMP in the hepatocyte and adipocyte (1, 4). Cyclic AMP is also involved in the release of insulin (19), and we have recently presented evidence indicating that the defective insulin release of the prediabetic may have its origin in a decreased ability of the beta cell to produce cyclic AMP (10). It seemed possible that, in the prediabetic subject, if the defect is not limited to the beta cell adenyl cyclase–cyclic AMP system, but is also present in the cyclases of liver and fat cells, this would result in a metabolic balance: on the one hand less insulin would be secreted and, on the other the increased glycogenolysis and lipolysis to be expected from insulin lack would be limited due to the decrease in cyclic AMP formation in liver and adipose tissue (for a detailed discussion of this topic see references 12 and 25).

The data from the present study do not support the hypothesis stated above. We were unable to demonstrate a reduction in the effectiveness of epinephrine in increasing blood glucose and plasma glycerol in prediabetic subjects. The mean values for blood glucose were not significantly different in the two groups of subjects, either when epinephrine was infused alone or when the glucose regulatory mechanisms were further stressed by the addition of a glucose infusion. Also plasma glycerol behaved in a similar manner in the two groups during the present experiments. There occurred marked inter-subject variations in the responses to epinephrine in both groups. Whether a similar study performed on a substantially larger number of subjects would be able to unmask subtler differences between the groups remains a matter of speculation. In this study only one dose of epinephrine was used. If this dose is a supramaximal one for lipid and glucose mobilization, complete dose-response studies in these two groups of subjects might be more suitable for detecting differences in sensitivity than the cross-section type of study presented here.

Its limitations kept in mind, this study suggests that, in prediabetes, there is no gross deficiency of the adenyl cyclase–cyclic AMP systems of liver and adipose tissue cells. Although the cyclic AMP system is common to almost all tissues, the specificity of this system is brought about by a cell membrane receptor or discriminator that couples the specific stimulus (hormones etc.) to adenyl cyclase (2). We have recently presented a hypothesis according to which the rapid insulin releasing effect of glucose is due to the interaction of glucose with a specific glucose receptor in the beta cell, resulting in stimulation of adenyl cyclase and production of cyclic AMP (13). We also postulated that the decreased cyclic AMP generation during glucose administration in prediabetic beta cells may be due to a defect in the above mentioned glucose receptor rather than to a derangement of the enzyme adenyl cyclase. We think that the findings of the present study are in keeping with such a concept.

The mechanisms that protect a prediabetic individual from developing manifest diabetes seem to be of a subtle nature and difficult to unravel with experimental procedures similar to the one used in the present paper. Biochemical studies on specimens from hepatic and adipose tissue from normal, prediabetic and diabetic subjects will be a necessary step in the elucidation of this problem.

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THE LIPID PATTERN IN NORMALS AND ATHEROSCLEROTICS

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Abstract. Blood lipid studies with lipoprotein typing a.m. Fredrickson were performed in 1 155 persons including 207 normals, 559 coronary heart disease patients, and 101 patients with atherosclerosis obliterans of the lower limbs. The results of lipoprotein typing were as follows. Type Normal: 43.2%, 54.8% and 64.8%. Type II: 43.9%, 32.3% and 20.3%. Type IV: 9.2% and 3.7% could not be classified. (The figures refer to upper cut-off points of normal cholesterol of 775, 300 and 325 mg/100 ml, respectively.) Triglyceride levels were distinctly correlated to body weight.

Epidemiologic studies linking blood lipids of population groups with the incidence of atherosclerotic disease have increased the interest in defining normal or desirable ranges of serum lipids. The introduction of the classification system of hyperlipidemias by the Fredrickson group in 1967 has been an additional stimulus to this interest, precipitating a wave of reports on lipoprotein patterns in people with and without clinical manifestations of atherosclerosis. The results of these studies indicate the possible existence of geographical differences in the lipid pattern, especially in patients with coronary heart disease. Thus, it is thought to be important that many centers report their results of the Fredrickson typing of hyperlipoproteinemias. Because of methodological difficulties in the use of the Fredrickson system, it is important for reasons of comparison of the results that the criteria of classification are precisely defined.

The purpose of this communication is to provide a survey of the clinical use of the Fredrickson classification system in the lipid clinic of a large general hospital in Oslo.

MATERIAL AND METHODS

The persons in the present report were studied between January 1968 and March 1970. The normals were medical students, physicians, nurses, and workers and employees of three industrial companies in Oslo. The coronary patients were both hospital and ambulatory patients, whereas the patients with intermittent claudication chiefly had been referred to the outpatient clinic for peripheral arterial insufficiency of Department VIII, Ullevaal Hospital.

Procedures included history and physical examination with weight and height measurements (8), and the referring physician provided a filled-in questionnaire in each case to enable us to ascertain the diagnosis and the possibility of exclusion of persons with diseases known to influence blood lipids such as diabetes, diseases of the thyroid and the liver, nephrotic syndrome and excessive alcohol ingestion. Deviations from usual food habits, such as carbohydrate restriction, low fat, and polyunsaturated-substituted diets, as well as the use of lipid reducing drugs were also registered.

All lipid determinations were made after a 1 14 hours overnight fast. Total abstinence from alcoholic beverages on the day before examination was recommended. Serum was inspected and characterized as clear, cloudy or milky and analyzed for total cholesterol (1), triglycerides (2, 5) and glucose (7). Glucose tolerance tests were not performed. Electrophoretic separation of lipoprotein was carried out by the method of Lees and Hatch (6) using hanging paper strips in Durrum cells. The strips were stained with Sudan Black B (4) and after visual inspection, classification of the lipoproteinemias was made according to the Fredrickson system (3).

Quantitation of the separated lipoproteins was tried by a visual grading of the relative dye uptake of the lipoprotein fraction, and a score system was established as apparent from Table IX.

Criteria for classification of lipoprotein pattern

Normal: Cholesterol < 275 (< 300, < 325 mg/100 ml). Triglycerides < 200 mg/100 ml.

Type I. As defined by Fredrickson et al. (3).

Type II Cholesterol ≥ 275 (≥ 100, ≥ 325). Triglycerides—normal or increased. Electrophoresis—distinct β-band; no or only slight and diffuse fraction in front of β-band. If distinct and separate pre-β-band, triglycerides < 200 mg/100 ml.

Type IV Cholesterol—normal or increased. Triglycerides

Table I. *Lipid pattern in normals and atherosclerotics Age and sex distribution*

	Males			Females			Total		
Age groups	No.	Horisont. (%)	Vertical (%)	No.	Horisont. (%)	Vertical (%)	No.	Horisont. (%)	Vertical (%)
20–29	66	72.5	7.8	25	27.5	8.1	91	100.0	7.9
30–39	95	83.3	11.2	19	16.7	6.2	114	100.0	9.9
40–49	204	81.3	24.1	47	18.7	15.3	251	100.0	21.7
50–59	331	70.1	39.1	141	29.9	45.8	472	100.0	40.9
60–64	151	66.5	17.8	76	33.5	24.7	227	100.0	19.7
Total	847	73.3	100.0	308	26.7	100.0	1 155	100.0	100.1

Table II. *Lipid pattern in normals and atherosclerotics Distribution by diagnosis*

	Males		Females		Total	
Diagnosis	No.	%	No.	%	N	%
Normals	173	71.1	34	28.9	207	100.0
CHD	427	76.4	132	23.6	559	100.0
ASO	77	76.2	24	23.8	101	100.0
Cerebr. vasc. dis.	18	56.3	14	43.8	32	100.1

> 200 mg/100 ml. Electrophoresis—distinct and separate pre-β-band.

Type V Type IV + chylomikron electrophoretically

Unclassified. Lipid patterns which could not be classified as Type I, II, IV or V

Results of lipoprotein-typing also given when the upper cut-off points of normal serum cholesterol were set to 300 and 325 mg/100 ml respectively

In total, 1 493 determinations of the lipid pattern were made in 1 331 persons during the 27-month period. I the present report statistical analysis has been limited to the first examination in men and women aged 20–64 in total 1 155 persons.

RESULTS

Table I presents age and sex distribution, and Table II the distribution of normals and of patients with certain specified atherosclerotic disease entities to be studied more closely in the following three articles. The cerebral vascular group has been excluded from further statistical analysis due to small numbers.

It should be kept in mind that a single person may be counted in more than one disease group, e.g. both in the coronary heart disease (CHD) group and in the group with atherosclerosis obliterans of the lower limbs (ASO)

Table III presents the mean values of total cholesterol, triglycerides and glucose in normals and in the disease groups.

Table IV presents the results of lipoprotein classification and the mean values of cholesterol, triglycerides and glucose in the lipoprotein types. Figures for lipoprotein types have been given for three cut-offs for upper normal cholesterol limits. According to the diagnostic criteria used, the lipoprotein pattern of 42 persons (3.7%) could not be classified.

Tables V and VI present the distribution of serum cholesterol and triglyceride levels in the lipoprotein types. Cholesterol below 275 mg/100 ml was found in 22 (20.7%) in Type IV whereas triglycerides higher than 200 mg/100 ml were encountered in 47 (9.3%) in Type II. Table VII shows the presence of tendinous xanthomas and

Table III. *Lipid pattern in normals and atherosclerotics. Means for blood cholesterol, triglycerides and glucose in normals and in disease groups*

	Normals (n = 207)			CHD (n = 559)			ASO (n = 101)			All (n = 1155)		
	Mean	S.D	S.E.	Mean	S.D	S.E.	Mean	S.D	S.E.	Mean	S.D	S.E.
Cholesterol	236	49	3.4	301	71	3.0	325	80	8.0	288	80	2.3
Triglycerides	88	53	3.7	161	164	6.9	180	204	20.3	144	151	4.5
Glucose	71	10	0.7	91	29	1.2	87	31	3.2	84	27	0.8

Table IV *Lipid pattern in normals and atherosclerotics. Mean values of blood cholesterol, triglycerides and glucose in Fredrickson lipoprotein types[a]. Both sexes combined*

	Lipoprotein type		Cholesterol			Triglycerides			Glucose		
	No.	%	Mean	S.D.	S.E.	Mean	S.D	S.E.	Mean	S.D.	S.E.
Normal	499	43.2	226	34	1.5	90	37	1.6	82	4	1.1
Type II	508	43.9	338	61	2.7	128	49	2.6	84	26	1.2
Type IV	106	9.2	337	103	10.0	434	355	34.5	94	38	3.8
Unclassified	42	3.7	307	89	13.7	241	96	14.8	87	40	6.6
Total	1155	100.0	288	80	2.3	144	151	4.4	84	27	0.8

[a] If cut-off points of normal cholesterol are set at 300 or 325 mg/100 ml, respectively, the distribution of lipoprotein types will be changed as follows. Type Normal: 633 (54.8 %) or 772 (66.8 %). Type II 374 (12.3 %) or 235 (20.3 %). Type IV and type Unclassified, figures unchanged.

of xanthelasmas in the lipoprotein types. Tendinous xanthomas were found in 8.1 and 4.8 % in Type II and Type IV, respectively. Eruptive xanthomas were not encountered.

Table VIII relates relative weight and lipoprotein type. Overweight occurred in 16.6 %, 33.7 % and 51.9 % in lipoprotein Types Normal, II and IV respectively. Overweight more than 20 % was found in 11.9 % and 14.7 % in Type Normal and Type II, respectively, in contrast to 34.9 % in Type IV.

Figs. 1 and 2 present the relation between cholesterol and triglyceride levels and relative weight. The cholesterol level shows a slight increasing trend with increasing relative weight, whereas there is a distinct correlation between relative weight and the triglyceride level.

Table IX presents the results of an attempt to quantitate the lipoproteins by visual grading of the relative dye uptake by means of a score system. The α-lipoprotein fraction was always visible on wet strips, but not always after drying. The occurrence of chylomicron in two persons in type unclassified was due to the fact that they erroneously were non-fasting.

It should be noted that score 2 for β-fraction was given in almost the same percentage in Type II and Type Normal, indicating that the classifica-

Table V *Lipid pattern in normals and atherosclerotics. Distribution of serum cholesterol levels in lipoprotein types. Both sexes combined*

	Lipoprotein types									
Cholesterol groups	Normal		Type II		Type IV		Unclassified		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
600-	0	0	2	0.4	4	3.8	0	0	6	0.5
575–599	0	0	2	0.4	0	0	0	0	2	0.2
525–574	0	0	11	2.2	1	0.9	1	2.4	13	1.1
475–524	0	0	8	1.6	4	3.8	1	2.4	13	1.1
425–474	0	0	18	3.5	5	4.7	3	7.1	26	2.3
375–424	0	0	54	10.6	8	7.5	4	9.5	66	5.7
325–374	0	0	140	27.6	27	25.5	5	11.9	172	14.9
275–324	0	0	273	53.8	35	33.0	11	26.2	319	27.5
225–274	284	56.9	0	0	15	14.2	11	26.2	310	26.9
200–224	113	22.6	0	0	3	2.8	3	7.1	119	10.3
150–199	83	16.6	0	0	3	2.8	2	4.8	88	7.6
149	19	3.8	0	0	1	0.9	1	2.4	21	1.8
Total	499	99.9	508	100.1	106	99.9	42	100.0	1155	99.9

Table VI. *Lipid pattern in normals and atherosclerotics. Distribution of serum triglyceride levels in lipoprotein types. Both sexes combined*

Triglyceride groups	Lipoprotein types: Normal No.	Normal %	Type II No.	Type II %	Type IV No.	Type IV %	Unclassified No.	Unclassified %	Total No.	Total %
2 000–	0	0	0	0	1	0.9	0	0	1	0.1
1 000–1 999	0	0	0	0	5	4.7	0	0	5	0.4
800–999	0	0	0	0	6	5.7	0	0	6	0.5
600–799	0	0	0	0	6	5.7	1	2.4	7	0.6
500–599	0	0	0	0	1	0.9	0	0	1	0.1
400–499	0	0	0	0	14	13.2	1	2.4	15	1.3
350–399	0	0	0	0	6	5.7	3	7 1	9	0 8
300–349	0	0	1	0.2	14	13.2	6	14.3	21	1.8
250–299	0	0	8	1.6	30	28.3	3	7 1	41	3.5
200– 49	0	0	38	7.5	23	21 7	15	35.7	76	6.6
150–199	39	7.8	101	19.9	0	0	7	16.7	147	12.7
100–149	134	26.9	206	40 6	0	0	6	14.3	346	30 0
50–99	276	55.3	151	29 7	0	0	0	0	427	37.0
–49	50	10.0	3	0.6	0	0	0	0	53	4.6
Total	499	100.0	508	100.1	106	100.0	42	100.0	1155	100 0

Table VII. *Lipid pattern in normals and atherosclerotics. Presence of xanthomas and xanthelasmas in lipoprotein types. Both sexes combined*

Lipoprotein type	No.	Xanthoma No.	Xanthoma %	Xanthelasmas No.	Xanthelasmas %
Normal	493	2	0.4	4	0.8
Type II	493	40	8.1	19	3.9
Type IV	105	5	4.8	2	1.9
Unclassified	40	0	0	4	10
Total	1131	47	4.2	29	2.6

24 not examined for xanthomas and xanthelasmas.

tion of the lipoprotein type should not be based on visual evaluation of dye intensity of electrophoretic strips only

DISCUSSION

The present report reviews the results of lipid determinations of 1 155 persons, aged 20–64. The younger age groups are underrepresented, and so are females, whereas coronary heart disease patients are by far the largest group. This skew age, sex and disease category distribution makes tests of significance between various groups uncertain, which is the reason why such tests have

Table VIII. *Lipid pattern in normals and atherosclerotics. Relative weight in lipoprotein types. Both sexes combined*

Lipoprotein type	Underweight (%) >20 No.	%	Underweight (%) 11–20 No.	%	Normal weight (%) ±10 No.	%	Overweight (%) 11–20 No.	%	21–30 No.	%	31–40 No.	%	41 50 No.	%	>50 No.	%	Not examin. No	%	Total No.	%
Normal	16	3.2	54	10.8	293	58.7	70	14.0	38	7.6	10	2.0	6	1.2	5	1.0	7	1.4	499	99.9
Type II	20	3.9	44	8.7	260	51.2	98	19.3	40	7.9	20	3 9	6	1.2	7	1.4	13	2.6	508	100.1
Type IV	1	0.9	4	3.8	46	43.4	18	17.0	16	15.1	10	9 4	6	5.7	5	4.7	0	0	106	100 0
Unclassified	0	0	2	4.8	19	45.2	9	21 4	6	14.3	1	2.4	1	2.4	0	0	4	9.5	42	100.0
Total	37	3.2	104	9.0	618	53.5	195	16.9	100	8.7	41	3.5	19	1.6	17	1.5	24	2.1	1155	100 0

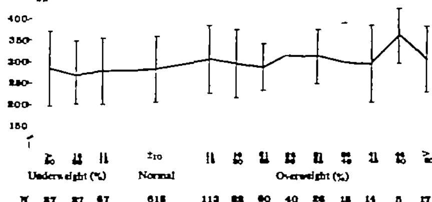


Fig 1 Lipid pattern in normals and atherosclerotics. Relative weight in relation to serum cholesterol.

largely been omitted in the present reports. Planned reports on extended material will permit us to make a more extensive use of significance tests.

The overall results of the present study include some persons with secondary hyperlipidemias due to diseases influencing the lipid pattern, such as diabetes, liver and thyroid disease, and in the CHD group some patients with an acute myocardial infarction in whom the blood was drawn more than two days after onset of symptoms, when the concentration of serum cholesterol and triglycerides may have changed considerably In the ensuing reports on blood lipids in the specified groups, normals, CHD and ASO patients, all persons with diseases, or being in a state known to influence blood lipids, have been excluded from analysis.

Types I, III and V have not been diagnosed. The diagnosis of Types I and V is not difficult, being based on the presence of chylomicron electrophoretically The diagnosis of Type III ("broad beta disease") cannot be made with any certainty on the basis of paper electrophoresis. It cannot be excluded that some Type III cases are hidden in the unclassified group, and possibly also in the Type II group.

It should be noted that the distinction between Type Normal and Type II entirely depends on the setting of normal serum cholesterol levels, while the diagnosis of Type IV is independent of the definition of the normal cholesterol range, being based on an elevated triglyceride level and on the presence of an electrophoretically distinct pre-β-lipoprotein band. In comparing results of lipoprotein typing of normals and in various disease entities from different research groups, the importance of the setting of normal upper cut

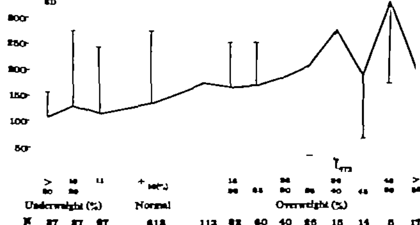


Fig 2. Lipid pattern in normals and atherosclerotics. Relative weight in relation to serum triglycerides.

lpid pattern in normals and atherosclerotics. Electrophoretic scores in lipoprotein types. Both sexes

Electrophoretic fractions	Score	Lipoprotein types: Normal No.	%	Type II No.	%	Type IV No.	%	Unclassified No.	%
Chylomicron	0	499	100.0	508	100 0	106	100.0	40	95.2
	1	0	0	0	0	0	0	2	4.8
β-fraction	0	0	0	0	0	0	0	0	0
	1	173	34.7	17	3.3	9	8.5	4	9.5
	2	319	63.9	361	71 1	79	74 5	32	76.2
	3	7	1.4	130	25.6	18	17.0	6	14.3
Distinct and sep. pre-β-fraction	0	488	97.8	485	95.5	0	0	35	83.3
	1	11	2.2	20	3.9	40	37 7	6	14.3
	2	0	0	3	0.6	47	44.3	1	2 4
	3	0	0	0	0	19	17.9	0	0
Unseparated pre-β- and broad β-fraction	0	427	85.6	357	70.3	97	91.5	13	31.0
	1	71	14.2	135	26.6	4	3.8	13	31.0
	2	1	0.2	16	3.1	5	4 7	13	31.0
	3	0	0	0	0	0	0	3	7 1
Total		499		508		106		42	

off points of serum cholesterol should be kept in mind. To make such comparison possible we have given figures for the lipoprotein typing at different settings of the upper normal cholesterol limit.

The classification system of the Fredrickson group, which is based on the determination of lipoprotein fractions, has proved to be useful However this research group has not claimed that their type system is perfect or final. The fact *that xanthomas and xanthelasmas, as in the present study* may be found in persons who should be typed normal according to their criteria seems unlogical.

The distinct correlation between triglyceride levels and body weight, also demonstrable in the present study has a therapeutic implication for *Type IV subjects, and confirms clinical* experience that weight reduction in overweight Type IV persons very often normalizes their blood lipids.

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BLOOD LIPIDS IN NORMALS

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Abstract. Lipid pattern has been studied in 207 (173 males and 34 females) healthy persons. Mean serum cholesterol and triglycerides were 202 and 61, 243 and 100, 256 and 93, 266 and 121, 273 and 109 mg/100 ml, respectively in the age groups 20–29, 30–39, 40–49, 50–59 and 60–64. With setting of an upper normal cholesterol limit at 275–300–325 mg/100 ml, respectively the percentage distribution of lipoprotein types was as follows. Type Normal: 79.2–87.4–93.7, Type II: 18.4–10.1–3.9, Type IV: 2.4–independent of the setting of the upper normal cholesterol limit.

Normal ranges of blood lipids in the sense of average values should not be confused with desirable values.

The present study reports the results of lipid analysis in persons thought to represent the normal healthy population, i.e. they were all without clinical symptoms of coronary heart disease (CHD), atherosclerosis obliterans of the lower limbs (ASO), and of cerebral vascular disease, and without conditions known to influence blood lipids.

MATERIAL AND METHODS

The report deals with the results of the first examination of normal persons aged 20–64 studied between January 68 and March 1970, and includes medical students, nurses, physicians, and workers and employees of three industrial companies in Oslo. Persons studied in the lipid clinic because of proved or suspected hyperlipidaemia have been excluded, even when atherosclerotic disease could not be diagnosed.

Methods for blood lipid analyses have previously been accounted for (5) and include fasting determination of total cholesterol, triglycerides, glucose, and paper lipoprotein electrophoresis, as well as classification of the lipoprotein type according to the system of the Fredrickson group.

RESULTS

A total of 207 normal persons were studied. Table I presents age and sex distribution. In Tables II–V age distribution of serum cholesterol and triglycerides is presented in males, females, and in both sexes combined. Tables VI–VII give the results of lipoprotein typing and mean values for blood lipids and glucose in various lipoprotein types. Abnormal lipid pattern was encountered in 20.8% of the normals with an upper normal cholesterol limit of 275 mg/100 ml. Table VIII presents the relation between relative weight and lipoprotein types.

DISCUSSION

The present material is too small to be of any great importance in defining lipid ranges in normals. However, the fact that reports on the classification of lipoprotein types in normals are still very rare warrants a presentation even of small materials. As in the patient groups (6, 7), there are far more men than women. Furthermore, higher age groups are underrepresented. However, it should be kept in mind that normalcy in the meaning of absence of atherosclerotic disease is a very difficult problem in higher age groups. Normalcy in this sense just means being without symptoms, a state which may suddenly change the next day or week after examination.

The results confirm earlier reports (1, 2, 3, 4, 6, 7, 8, 9) and show increasing lipid values with age up to about 60 years. Some 20% of the normals present with an abnormal lipoprotein pattern, Type II being by far the larger group with the setting of upper normal cholesterol limit at 275 mg/100 ml. However, it needs to be em-

Table I. *Blood lipids in normals. Age and sex distribution*

	Males			Females			Total		
Age groups	No.	Horisont. (%)	Vertical (%)	No.	Horisont. (%)	Vertical (%)	No.	Horisont. (%)	Vertical (%)
20–29	50	70.4	28.9	21	29.6	61.8	71	100.0	34.3
30–39	59	93.7	34.1	4	6.3	11.8	63	100.0	30.4
40–49	38	88.4	22.0	5	11.6	14.7	43	100.0	20.8
50–59	17	81.0	9.8	4	19.0	11.8	21	100.0	10.1
60–64	9	100.0	5.2	0	0	0	9	100.0	4.3
Total	173	83.6	100.0	34	16.4	100.0	207	100.0	99.9

Table II. *Blood lipids in normals. Serum cholesterol levels in age groups. Both sexes combined*

Cholesterol groups	Age groups 20–29	30–39	40–49	50–59	60–64	Total
400–	0	1	0	0	0	1
375–399	0	0	0	0	0	0
350–374	0	1	0	0	0	1
325–349	0	1	1	3	1	6
300–324	1	3	6	1	2	13
275–299	2	7	7	4	1	21
250–274	7	13	8	5	2	35
225–249	12	13	12	5	1	43
200–224	11	14	7	2	2	36
175–199	21	7	2	1	0	31
150–174	8	3	0	0	0	11
–149	9	0	0	0	0	9
Total	71	63	43	21	9	207
Mean	202	243	256	266	273	236
Range	126–306	154–418	196–348	198–344	220–344	126–418
S.D	40	47	37	41	45	49
S.E.	4.8	5.9	5.6	8.9	15.1	3.9

Table III. *Blood lipids in normals. Mean cholesterol values in age groups*

Age	No.	Serum cholesterol Mean	Range	S.D	S.E.
Males					
20–29	50	196	126–306	40	5.8
30–39	59	245	154–418	47	6.2
40–49	38	258	196–348	39	6.2
50–59	17	262	212–328	32	7.8
60–64	9	273	220–344	45	15.1
Total	173	237	126–418	50	3.8
Females					
20–29	21	217	145–294	35	7.6
30–39	4	219	169–255	41	20.5
40–49	5	245	216–264	18	8.1
50–59	4	283	198–344	71	35.3
60–64	0	—	—	—	—
Total	34	229	145–344	43	7.4

Table V. *Blood lipids in normals. Mean triglyceride values in age groups*

Age	No.	Serum triglycerides Mean	Range	S.D	S.E.
Males					
20–29	50	63	31–158	28	4.0
30–39	59	103	38–465	71	9.2
40–49	38	96	47–230	36	5.8
50–59	17	122	68–337	64	15.6
60–64	9	109	59–177	41	13.7
Total	173	92	31–465	55	4.2
Females					
20–29	21	54	27–94	20	4.2
30–39	4	54	44–68	10	5.0
40–49	5	72	43–118	29	12.8
50–59	4	120	100–147	20	9.8
60–64	0	—	—	—	—
Total	34	64	27–147	29	5.0

Table IV *Blood lipids in normals. Serum triglyceride levels in age groups. Both sexes combined*

Triglyceride groups	Age groups 20–29	30–39	40–49	50–59	60–64	Total
500-	0	0	0	0	0	0
300–499	0	2	0	1	0	3
250–299	0	0	0	0	0	0
200–249	0	1	1	0	0	2
175–199	0	2	1	0	1	4
150–174	1	2	0	3	0	6
125–149	2	4	5	4	3	18
100–124	3	13	6	4	1	27
75–99	9	13	17	7	1	47
50–74	30	20	11	2	3	66
25–49	26	6	2	0	0	34
24	0	0	0	0	0	0
Total	71	63	43	21	9	207
Mean triglycerides	61	100	93	121	109	88
Range	27–158	38–465	43–230	68–337	59–177	27–465
S.D.	26	69	36	58	41	53
S.E.	3.1	8.7	5.4	12.7	13.7	3.7

Table VI. *Blood lipids in normals. Fredrickson lipoprotein types by age and sex*

Age groups / Lipoprotein types	20–29		30–39		40–49		50–59		60–64		All ages			
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	Total*	%
Normal	49	19	45	4	25	5	11	1	5	0	135	29	164	79.2
Type II	1	2	11	0	12	0	5	3	4	0	33	5	38	18.4
Type IV	0	0	3	0	1	0	1	0	0	0	5	0	5	2.4
Unclassified	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	50	21	59	4	38	5	17	4	9	0	173	34	207	100.0

* If the cut-off points of normal serum cholesterol are set at 300 and 325 mg/100 ml, respectively the distribution of lipoprotein types will be changed. Type Normal: 181 (87.4%) and 194 (93.7%). Type II: 21 (10.1%) and 8 (3.9%). Type IV and type Unclassified, figures unchanged.

Table VII. *Blood lipids in normals. Mean values for blood cholesterol, triglycerides and glucose in lipoprotein types. Both sexes combined*

	Lipoprotein type		Cholesterol			Triglycerides			Glucose		
	No.	%	Mean	S.D.	S.E.	Mean	S.D.	S.E.	Mean	S.D.	S.E.
Normal	164	79.2	217	34	2.7	74	30	2.3	71	10	0.8
Type II	38	18.4	307	30	4.9	112	39	6.3	70	10	1.6
Type IV	5	2.4	289	24	10.7	325	100	44.9	72	8	3.5
Unclassified	0	0	—	—	—	—	—	—	—	—	—
Total	207	100.0	236	49	3.4	88	53	3.7	71	10	0.7

Table I. *Blood lipids in normals. Age and sex distribution*

	Males			Females			Total		
Age groups	No.	Horisont. (%)	Vertical (%)	No.	Horisont. (%)	Vertical (%)	No.	Horisont. (%)	Vertical (%)
20–29	50	70.4	28.9	21	29 6	61.8	71	100.0	34.3
30–39	59	93 7	34 1	4	6 3	11.8	63	100.0	30.4
40–49	38	88.4	22.0	5	11.6	14.7	43	100.0	20.8
50–59	17	81.0	9.8	4	19 0	11.8	21	100.0	10.1
60–64	9	100.0	5.2	0	0	0	9	100 0	4.3
Total	173	83.6	100 0	34	16.4	100.0	207	100.0	99.9

Table II. *Blood lipids in normals. Serum cholesterol levels in age groups Both sexes combined*

Cholesterol groups	Age groups 20–29	30–39	40–49	50–59	60–64	Total
400–	0	1	0	0	0	1
375–399	0	0	0	0	0	0
350–374	0	1	0	0	0	1
325–349	0	1	1	3	1	6
300–324	1	3	6	1	2	13
275–299	2	7	7	4	1	21
250–274	7	13	8	5	2	35
225–249	12	13	12	5	1	43
200–224	11	14	7	2	2	36
175–199	21	7	2	1	0	31
150–174	8	3	0	0	0	11
–149	9	0	0	0	0	9
Total	71	63	43	21	9	207
Mean	202	243	256	266	273	236
Range	126–306	154–418	196–348	198–344	220–344	126–418
S.D	40	47	37	41	45	49
S.E.	4 8	5.9	5 6	8.9	15.1	3.9

Table III. *Blood lipids in normals. Mean cholesterol values in age groups*

	Serum cholesterol				
Age	No.	Mean	Range	S.D	S.E.
Males					
20–29	50	196	126–306	40	5.8
30–39	59	245	154–418	47	6.2
40–49	38	258	196–348	39	6.2
50–59	17	262	212 328	32	7.8
60–64	9	273	220–344	45	15.1
Total	173	237	126–418	50	3.8
Females					
20–29	21	217	145–294	35	7 6
30–39	4	219	169–255	41	20.5
40–49	5	245	216–264	18	8 1
50–59	4	283	198–344	71	35.3
60–64	0	—	—	—	—
Total	34	229	145–344	43	7 4

Table V *Blood lipids in normals. Mean triglyceride values in age groups*

	Serum triglycerides				
Age	No.	Mean	Range	S.D.	S E.
Males					
20–29	50	63	31–158	28	4.0
30–39	59	103	38–465	71	9.2
40–49	38	96	47–230	36	5.8
50–59	17	122	68–337	64	15 6
60–64	9	109	59–177	41	13 7
Total	173	92	31–465	55	4 2
Females					
20–29	21	54	27–94	20	4 2
30–39	4	54	44–68	10	5.0
40–49	5	77	43–118	29	12.8
50–59	4	120	100–147	20	9.8
60–64	0	—	—	—	—
Total	34	64	27–147	29	5 0

Table IV *Blood lipids in normals Serum triglyceride levels in age groups Both sexes combined*

Triglyceride groups	Age groups 20–29	30–39	40–49	50–59	60–64	Total
300–	0	0	0	0	0	0
300–499	0	2	0	1	0	3
250–299	0	0	0	0	0	0
200–249	0	1	1	0	0	
175–199	0	2	1	0	1	4
150–174	1	2	0	3	0	6
125–149	2	4	5	4	3	18
100–124	3	13	6	4	1	77
75–99	9	13	17	7	1	47
50–74	30	20	11	2	3	66
25–49	26	6	2	0	0	34
24	0	0	0	0	0	0
Total	71	63	43	21	9	207
Mean triglycerides	61	100	93	121	109	88
Range	27 158	38–465	43–230	68–337	59–177	27–465
S.D	26	69	36	58	41	53
S.E.	3.1	8.7	5.4	12.7	13 7	3 7

Table VI. *Blood lipids in normals. Fredrickson lipoprotein types by age and sex*

Age groups / Lipoprotein types	20–29 ♂	20–29 ♀	30–39 ♂	30–39 ♀	40–49 ♂	40–49 ♀	50–59 ♂	50–59 ♀	60–64 ♂		All ages		Total*	%
Normal	49	19	45	4	25	5	11	1	5	0	135	29	164	79 2
Type II	1	2	11	0	12	0	5	3	4	0	33	5	38	18 4
Type IV	0	0	3	0	1	0	1	0	0	0	5	0	5	2.4
Unclassified	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	50	21	59	4	38	5	17	4	9	0	173	34	207	100.0

If the cut-off points of normal serum cholesterol are set at 300 and 325 mg/100 ml, respectively the distribution of lipoprotein types will be changed. Type Normal 181 (87.4%) and 194 (93.7%). Type II 21 (10.1 %) and 8 (3.9%). Type IV and type Unclassified, figures unchanged.

Table VII. *Blood lipids in normals. Mean values for blood cholesterol, triglycerides and glucose in lipoprotein types. Both sexes combined*

	Lipoprotein type N	%	Cholesterol Mean	S.D.	S.E.	Triglycerides Mean	S.D	S.E.	Glucose Mean	S.D.	S.E.
Normal	164	79.2	217	34	2.7	74	30	2.3	71	10	0.8
Type II	38	18 4	307	30	4 9	112	39	6 3	70	10	1.6
Type IV	5	2.4	289	24	10.7	125	100	44 9	72	8	3.5
Unclassified	0	0	—	—	—	—	—	—	—	—	—
Total	207	100 0	236	49	3.4	88	53	3.7	71	10	0.7

Table VIII *Blood lipids in normals Relative weight in lipoprotein types Both sexes combined*

Lipoprotein type*	Underweight (%)		Normal weight (%)	Overweight (%)			Total
	>20	11 20	±10	11 20	21 30	31–40	
Normal	1	18	121	19	5	0	164
Type II	1	4	27	4	1	1	38
Type IV	0	0	2	2	1	0	5
Total	2	22	150	25	7	1	207
Per cent	1.0	10 6	72.5	12.1	3.4	0.5	100.1

phasized that the definition of normal cholesterol ranges, largely *influences the results of lipoprotein typing*. Thus, with an upper normal cholesterol limit of 325 (Table VI) the prevalence of Types II and IV is almost the same. This upper normal cholesterol limit, however seems far too high in a group of individuals of whom about two-thirds are *less than 40 years of age*.

To be able to draw more certain conclusions about the prevalence of various lipoprotein types in the healthy population, studies of larger groups are mandatory Such extended studies are planned.

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BLOOD LIPIDS IN PATIENTS WITH CORONARY HEART DISEASE

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Abstract. The study reports cholesterol, triglyceride and glucose values in 420 coronary heart disease patients, aged 30–64. Patients on lipid lowering diet or drugs, or being in [illegible] state, as well as having diseases, known to influence lipid levels, were excluded. The percentage distribution of Fredrickson lipoprotein types was as follows. Type Normal: 25.5–37.8–54.0. Type II: 59.0–46.7–30.5. Type IV 11.7%. Unclassified 3.8% independent of upper normal cholesterol limit. The figures refer to [illegible] setting of upper normal cholesterol limit of 275, 300 and 325 mg/100 ml, respectively. Overweight was more common in Type IV than in Type Normal and Type II.

Coronary heart disease (CHD) has been associated with a number of physiologic abnormalities, of which elevated blood lipids and hypertension have been most clearly defined.

Recent studies reporting prevalence data on lipoprotein types in CHD indicate geographical differences in the occurrence of pro-β-lipoproteins and of Type IV in the Fredrickson classification system. However different results may also be due to different methods and to the fact that the definition of the constitutional, or food-induced, Type II entirely depends on the setting of the upper normal cholesterol limit.

The present study reports blood lipid levels and lipoprotein types in CHD patients. For the purpose of comparison, results with different cut offs for normal cholesterol have been given.

MATERIAL AND METHODS

All patients in the present report had had [illegible] diagnosis of recent or previous myocardial infarction or of the syndrome of angina pectoris. The majority [illegible] are hospitalized in the medical departments of Ullevaal Hospital, and some were ambulatory patients attending the outpatient clinics of the hospital.

Procedures, laboratory methods and criteria for lipoprotein typing have been explained in [illegible] preceding paper (9).

Table I presents age and sex distribution. Totally 559 CHD patients [illegible] are studied. Excluded from further analysis are 139 patients for the following reasons:

(*a*) patients [illegible] with acute myocardial infarction in whom the difference between the date of the blood sampling and the date of onset of symptoms exceeded two days, in total 60 patients,

(*b*) patients with [illegible] diagnosed diabetes, thyroid and liver disease, nephrotic syndrome and alcoholism;

(*c*) patients known to be on strict low-fat or polyunsaturates-substituted diets, as [illegible] well as on carbohydrate restriction;

(*d*) patients on lipid-reducing drugs.

Thus, the present study deals with 420 patients [illegible] males and 102 females.

RESULTS

Tables II–V present separately and [illegible] the cholesterol and triglyceride data in [illegible] with and without acute myocardial [illegible] cholesterol level is about the [illegible] groups, whereas triglycerides [illegible] in younger male patients. In [illegible] sented the results of lipoprotein [illegible] to the Fredrickson system [illegible] upper normal limit of [illegible] influences the figures for [illegible] mal. However the [illegible] Type Unclassified is [illegible] cut-off point of [illegible] presents the lipoprotein [illegible] mal cholesterol [illegible] and female coronary [illegible] acute myocardial [illegible] bined, and in [illegible] levels have [illegible]

In Table IV [illegible]

Table I. *Blood lipids in patients with CHD Age and sex distribution*

	Males			Females			Total		
Age groups	No.	Horizont. (%)	Vertical (%)	No.	Horizont. (%)	Vertical (%)	No.	Horizont. (%)	Vertical (%)
20–29	0	0	0	0	0	0	0	0	0
30–39	9	100.0	2.1	0	0	0	9	100.0	1.6
40–49	95	87.2	22.2	14	12.8	10.6	109	100.0	19.5
50–59	219	76.3	51.3	68	23.7	51.5	287	100.0	51.3
60–64	104	67.5	24.4	50	32.5	37.9	154	100.0	27.5
Total	427	76.4	100.0	132	23.6	100.0	559	100.0	99.9
Excluded	109	78.4	25 1	30	21.6	22.7	139	100.0	24.9
Total	318	75.7	74.9	102	24.3	77.3	420	100.0	75 1

lipoprotein types. It should be noted that in Type Normal and Type II only 15.9 and 13.3% respectively were more than 20% overweight, in contrast to 38 8% in Type IV

DISCUSSION

The instability of blood lipid levels after infarction has been noted by several investigators and warrants care in using such data for establishing premorbid lipid ranges (4 6, 8, 10 13). During the first week after an infarction Dodds and Mills (2) found a decrease in total cholesterol and β-lipoproteins, whereas the triglyceride-rich pre-β-lipoproteins showed an increase. These changes would tend to increase the prevalence of Type IV whereas Type II figures will decrease. Dodds and Mills believe that the cholesterol-rich β-lipoproteins determined within 24 hours after infarction are probably similar to the levels before the attack.

In the present study patients have been excluded when blood was drawn later than two days after onset of symptoms. Nevertheless results from patients with and without acute myocardial infarction have been kept separate to test the

Table II. *Blood lipids in patients with CHD Serum cholesterol levels in age groups. Both sexes combined*

A. Acute myocardial infarction. B Not acute myocardial infarction

Age groups	30–39		40–49		50–59		60–64		Total		Total
Cholesterol groups	A	B	A	B	A	B	A	B	A	B	A+B
600–	0	0	0	0	0	0	0	1	0	1	1
575–599	0	0	0	0	0	1	0	0	0	1	1
525–574	0	0	0	1	0	2	1	1	1	4	5
475–524	0	0	0	2	0	2	0	0	0	4	4
425–474	0	0	0	1	3	3	1	3	4	7	11
375–424	0	0	1	8	6	8	5	5	12	21	33
325–374	2	1	6	11	27	30	7	11	42	53	95
275–324	1	3	16	14	32	38	23	21	72	76	148
225–274	1	0	11	8	34	19	9	17	55	44	99
200–224	0	0	4	0	6	3	2	1	12	4	16
150–199	0	0	0	0	3	1	2	0	5	1	6
149	0	0	0	0	0	0	0	1	0	1	1
Total	4	4	38	45	111	107	50	61	203	217	420
Mean	304	315	288	338	297	324	307	313	298	324	311
Range	232	288	202	234	180	196	186	136	180	136	136
	351	372	391	560	467	578	560	602	560	602	602
S.D	57	39	45	71	56	70	64	77	56	72	66
S.E.	28.2	19.3	7.3	10.5	5.3	6.8	9 1	9.9	3.9	4 9	3.2

Table III. *Blood lipids in patients with CHD Mean cholesterol values in age groups*

A. Acute myocardial infarction. B Not acute myocardial infarction

Age	No.	Mean	Range	S.D	S.E.
		Serum cholesterol			
Males					
30–39	8	310	232 377	45	16 0
40–49	73	312	202 560	65	7.6
50–59	164	307	180–578	62	4.9
60–64	73	287	136–437	52	6.1
Total	318	304	136–578	61	3 4
A	166	293	180–467	52	4.1
B	152	315	136–578	67	5.4
Females					
40–49	10	340	244–431	60	18.9
50–59	54	322	198–555	71	9.6
60–64	38	355	240–602	82	13.4
Total	102	336	198–602	75	7 4
A	37	320	198–560	67	11 1
B	65	345	236–602	79	9.7

Table V. *Blood lipids in patients with CHD Mean triglyceride values in age groups*

A: Acute myocardial infarction. B Not acute myocardial infarction

Age	No.	Mean	Range	S D	S.E.
		Serum triglycerides			
Males					
30–39	8	263	100–800	238	84 0
40–49	73	194	50–995	132	15 6
50–59	164	145	20–960	95	7 4
60–64	73	153	49–975	128	15 0
Total	318	155	20–995	118	6.6
A	166	150	20–995	140	10 8
B	152	159	50–800	89	7
Females					
40–49	10	117	68–202	49	15 5
50–59	54	203	51 1 870	293	39 8
60–64	38	153	60–445	100	16.[illegible]
Total	102	176	51–1 870	223	22.1
A	37	119	53–297	52	8.5
B	65	208	51–1 870	277	33 7

validity of these procedures. A trend toward lower cholesterol values in acute myocardial infarction is revealed, indicating that some reduction may have taken place. The triglyceride levels, however show no definite difference in coronary patients with and without myocardial infarction. Statistical methods to test the validity of such use of data from patients with and without acute coronary attacks have been omitted owing to small numbers and skew distribution of lipid values. A

Table IV *Blood lipids in patients with CHD Serum triglyceride levels in age groups. Both sexes combined*

A. Acute myocardial infarction. B Not acute myocardial infarction

Age groups / Triglyceride groups	30–39		40–49		50–59		60–64		Total		Total
	A	B	A	B	A	B	A	B	A	B	A + B
1 000–1 999	0	0	0	0	0		0	0	9	2	2
800–999	0	1	1	0	1	0	1	0	3	1	4
500–799	0	0	1	0	0	2	0	0	1	2	3
400–499	0	0	0	0	1	1	1	2	2	3	5
350–399	1	0	1	1	0	0	1	1	3	2	5
300–349	0	0	1	0	0	3	0	2	1	5	6
250–299	1	0	2	2	3	10	3	2	9	14	23
200–249	0	0	1	8	4	18	3	5	8	31	39
150–199	1	0	8	5	17	14	7	12	33	31	64
100–149	1	3	12	13	39	38	15	18	67	72	139
50–99	0	0	11	16	42	19	18	19	71	54	125
–49	0	0	0	0	4	0	1	0	5	0	5
Total	4	4	38	45	111	107	50	61	203	217	420
Mean	229	298	175	143	125	195	157	150	144	174	160
Range	100	108	50	61	20	51	49	50	70	50	20
	383	800	995	381	960	1 870	975	465	995	1 870	1 870
S.D	128	335	171	68	98	213	146	92	129	167	150
S.E.	63 8	167 7	27.7	10.1	9.3	20.6	20 6	11 7	9.0	11.3	7.3

Table VI. *Blood lipids in patients with CHD Fredrickson lipoprotein types by different upper limits of serum cholesterol. Both sexes combined*

Lipoprotein type	Upper normal cholesterol limit (mg 100 ml)					
	275		300		325	
	No.	%	No.	%	No.	%
Normal	107	25.5	159	37.8	227	54.0
Type II	48	59.0	196	46.7	128	30.5
Type IV	49	11 7	49	11.7	49	11.7
Unclassified	16	3.8	16	3.8	16	3.8
Total	420	100.0	420	100.0	420	100 0

planned extension of the material will probably settle such methodological problems with more certainty

The cholesterol and triglyceride values obtained in the present study are very much the same as previously reported on blood lipids in coronary patients in Oslo (11).

The prevalence of Type IV is lower in the present study than reported by others (3 4 7). In a recent review Gjone (5) reports partly unpublished results from Sweden, Denmark and Norway to the effect that an increase in the pre beta fraction was found in some 30% of coronary patients. In this connection it should be noted that the Fredrickson classification system allows some increase in the pre-beta fraction in Type II. Furthermore, in Type IV triglycerides should be elevated. In our study Type IV has not been diagnosed unless the triglycerides have exceeded 200 mg/100 ml and there has been a distinct and separate pre-beta band electrophoretically Our results are very much like those of Solberg (12) who, in a mixed patient material in Aker Hospital, Oslo, found a Type II/Type IV ratio of 5 It should be noted that Aker and Ulleval Hospitals serve the same population.

Methodological and classification differences may be responsible for the different results of lipoprotein typing. However geographical differences cannot be excluded.

The triglyceride levels observed in the present study are higher in males in the age groups 30–39 and 40–49 than in the age group 50–64 (Tables IV–V). However it should be noted that the age group 30–39 only includes 8 patients, of whom not more than 3 had triglyceride values above 250. Nevertheless, the male triglyceride level in age group 40–49 is also distinctly higher than in the age group 50–64 This observation confirms previous findings by Carlson (1), who has drawn attention to a tendency of elevated triglyceride levels in young males with CHD In females we found no certain triglyceride age trend.

Table VII. *Blood lipids in patients with CHD Presence of xanthomas and xanthelasmas in lipoprotein types. Four male patients and one female not examined*

A. Acute myocardial infarction. B Not acute myocardial infarction

	Lipoprotein type						Xanthoma		Xanthelasma	
	A		B		A – B		A + B		A + B	
	No.	Vertical (%)	No.	Vertical ( )	No.	Vertical ( )	No.	Horizont. (%)	No.	Horizont. (%)
Males										
Normal	55	33.1	31	20 4	86	27 0	0	—	0	—
Type II	86	51 8	94	61.8	180	56.6	12	6.7	6	3 3
Type IV	18	10.8	20	13.2	38	11.9	3	7 9	1	.6
Unclassified	7	4.2	7	4.6	14	4 4	0	—	1	7 1
Total	166	99.9	152	100.0	318	99.9	15	4 7	8	2.5
Females										
Normal	11	29 7	10	15.4	21	20.6	0	—	1	4.8
Type II	26	70.3	42	64.6	68	66.7	7	10 3		2.9
Type IV	0	0	11	16.9	11	10.8	1	9 1	0	—
Unclassified	0	0	2	3.1		2.0	0	—	0	—
Total	37	100.0	65	100.0	102	100.1	8	7.8	3	2.9

Table VIII. *Blood lipids in patients with CHD. Mean values for blood cholesterol, triglycerides and glucose in lipoprotein types*

	Lipoprotein type		Cholesterol			Triglycerides			Glucose		
	No	%	Mean	S.D.	S.E.	Mean	S.D.	S.E.	Mean	S.D.	S.E.
	Males										
Normal	86	27.0	239	25	2.7	97	38	4.1	92	27	[illegible].9
Type II	180	56.6	333	52	3.9	133	49	3.6	89	26	1.9
Type IV	38	11.9	311	44	7.2	355	219	35.6	91	21	3.4
Unclassified	14	4.4	302	65	17.5	[illegible]37	88	23.4	81	15	3.9
Total	318	99.9	304	61	3.4	155	118	6.6	90	25	1.4
	Females										
Normal	21	20.6	290	18	4.0	110	42	9.2	95	34	7.6
Type II	68	66.7	356	61	7.3	124	48	5.8	92	31	3.8
Type IV	11	10.8	383	104	31.3	616	492	148.4	75	12	4.1
Unclassified	2	2.0	303	41	29.0	212	16	11.5	77	4	3.0
Total	102	100.1	336	75	7.4	175	223	22.0	91	31	3.1

Table IX. *Blood lipids in patients with CHD. Relative weight in lipoprotein types. Both sexes combined*

Lipo-protein type	Underweight (%)				Normal weight (%)		Overweight (%)										Not exam.		Total	
	>20		11–20		±10		11–20		21–30		31–40		41–50		>50					
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	N	%	No.		No		No.	
Normal	2	1.9	9	8.4	57	53.3	21	19.6	11	10.3	2	1.9	1	0.9	3	2.8	1	0.9	107	100
Type II	7	2.8	19	7.7	130	52.4	53	21.4	19	7.7	12	4.8	1	0.4	1	0.4	6	2.4	48	100
Type IV	0	0	1	2.0	19	38.8	10	20.4	10	20.4	7	14.3	2	4.1	0	0	0	0	49	100
Unclassif.	0	0	0	0	8	50.0	5	31.3	3	18.8	0	0	0	0	0	0	0	0	16	100
Total	9	2.1	29	6.9	214	51.0	89	21.2	43	10.2	1	5.0	4	1.0	4	1.0	7	1.7	420	100

The demonstration of an increased prevalence of overweight in coronary patients with Type IV has implications for the treatment of that disorder.

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BLOOD LIPIDS IN PATIENTS WITH ATHEROSCLEROSIS OBLITERANS OF THE LOWER LIMBS

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Abstract. Blood cholesterol, triglycerides and glucose determinations, as well as paper electrophoretic separation of lipoproteins, were performed in 85 patients aged 30–64 (67 males and 18 females) with atherosclerosis obliterans of the lower limbs. Mean fasting cholesterol, triglyceride and glucose values were 322, 183 and 81 mg/100 ml, respectively. With setting of the upper normal cholesterol limits of 275, 300, and 325 mg/100 ml the percentage distribution of the Fredrickson lipoprotein types was as follows. Type Normal: 27.1, 37.7 and 49.4. Type II: 55.3, 44.7 and 32.9. Type IV was diagnosed in 12.9% and 4.7% could not be classified. Blood lipid and glucose levels have been given in different lipoprotein types. Only 28% of the patients were overweight.

In contrast to coronary heart disease (CHD) reports on the lipid pattern in patients with atherosclerosis obliterans of the lower limbs (ASO) have been relative few. Solvaag (4) studied blood cholesterol in 450 patients of all ages with ASO finding 41.6% with cholesterol values above 300 mg/100 ml and a mean cholesterol value of 293 mg/100 ml. To our knowledge results of the Fredrickson lipoprotein typing in patients with atherosclerosis obliterans of the lower limbs have not yet been published in Scandinavia. This study presents blood lipid values and lipoprotein types in such patients.

MATERIAL AND METHODS

The majority of the patients in the present study attended the special outpatient clinic for peripheral vascular insufficiency in Dept. VIII, Ullevaal Hospital. Only few patients had been hospitalized. The patients had been examined after standardized diagnostic procedure to establish the diagnosis of peripheral arterial insufficiency. Procedures and methods for blood lipid determination as well as criteria for lipoprotein typing have previously been described (2).

Table I presents age and sex distribution. Of the 101 patients studied, 16 were excluded for the following reasons: (*a*) patients with diagnoses of diabetes, thyroid and liver diseases, nephrotic syndrome and alcoholism, (*b*) patients known to be on low fat or polyunsaturates-substituted diets, as well as on carbohydrate restriction, (*c*) patients on lipid-reducing drugs.

Thus the present report deals with 85 patients, 67 males and 18 females.

RESULTS

Tables II–VI present cholesterol and triglyceride values in males and females. The cholesterol level is about the same in all age groups, whereas triglycerides in males show an increasing trend with age. The results of the lipoprotein typing at different cut-offs of normal serum cholesterol are given in Table VI showing a prevalence of lipoprotein types very much the same as in CHD patients (3).

Table VII presents blood lipid and glucose levels, and the occurrence of tendinous xanthomas and of xanthelasmas in different lipoprotein types. Data for the relation between lipoprotein types and relative weight have been given in Table VIII. The percentage with normal weight is the same as in CHD patients. It should be noted that 17.7% of the ASO patients were underweight, and only one fourth overweight.

DISCUSSION

The Framingham study (1) reports a pronounced increase of risk of atherosclerosis obliterans of the lower limbs (ASO) in persons with coronary heart disease (CHD), suggesting a common underlying basis. This theory is supported by the fact

Table I. *Blood lipids in patients with ASO Age and sex distribution*

Age groups	Males (No.)	Females (No.)	Total (No.)
20–39	0	0	0
30–39	3	1	4
40–49	16	4	20
50–59	38	10	48
60–64	20	9	29
Total	77	24	101
Excluded	10	6	16
Total	67	18	85

Table II. *Blood lipids in patients with ASO Serum cholesterol levels in age groups Both sexes combined*

Cholesterol groups	Age groups 30–39	40–49	50–59	60–64	Total
600–	0	0	0	1	1
475–599	0	0	1	0	1
425–474	0	1	3	1	5
375–424	0	2	9	1	12
325–374	0	4	11	7	22
275–324	1	6	6	8	21
225–274	0	4	8	6	18
200–224	1	0	2	0	3
199	0	0	2	0	2
Total	2	17	42	24	85
Mean	290	320	327	320	322
Range	210–289	246–454	184–480	228–602	184–602
S.D	56	54	73	81	71
S.E.	39.5	13.2	11.2	16.4	7 7

Table III. *Blood lipids in patients with ASO Mean cholesterol values in age groups*

Age groups	No.	Serum cholesterol Mean	Range	S.D	S.E.
Males					
30–39	1	289	—	—	—
40–49	13	314	246–454	55	15.2
50–59	35	333	184–440	67	11.3
60–64	18	300	228–437	57	13.5
Total	67	320	184–454	62	7 6
Females					
30–39	1	210	—	—	—
40–49	4	338	286–396	56	28.1
50–59	7	298	190–480	98	37 1
60–64	6	381	290–602	113	46.1
Total	18	330	190–602	100	23.5

that the principle hazard for patients with ASO derives from an increased risk of coronary heart disease rather than from the consequences of impaired circulation to the limb In the Selvaag study in Oslo (4) the coronary risk in ASO patients was increased when serum cholesterol exceeded 275 mg/100 ml.

In the present study the mean cholesterol and triglyceride levels in male ASO patients are very much the same as found in the CHD patients (3) However unlike the CHD triglyceride levels the male ASO triglycerides tend to increase with

Table IV *Blood lipids in patients with ASO Serum triglyceride levels in age groups. Both sexes combined*

Triglyceride groups	Age groups 30–39	40–49	50–59	60–64	Total
1000–1999	0	0	1	0	1
500–999	0	0	0	1	1
400–499	0	1	0	3	4
350–399	0	1	0	0	1
300–349	0	1	1	0	2
250–299	0	0	2	0	2
200–249	0	1	7	1	9
150–199	0	2	5	4	11
100–149	1	5	17	7	30
99	1	6	9	8	24
Total	2	17	42	24	85
Mean	83	164	188	194	183
Range	54–113	57–488	52 1 870	65–813	52–1 870
S.D	42	123	273	179	220
S.E.	29.5	29 7	42.2	36.6	23.9

Table V *Blood lipids in patients with ASO Mean triglyceride values in age groups*

Age groups	No.	Serum triglycerides Mean	Range	S.D	S.E.
Males					
30–39	1	113	—	—	—
40–49	13	138	57 390	102	28.2
50–59	35	148	52–333	68	11.5
60–64	18	179	65–813	184	43.3
Total	67	154	52–813	115	14.1
Females					
30–39	1	54	—	—	—
40–49	4	247	138–488	163	81 4
50–59	7	391	83–1870	654	247 1
60–64	6	241	78–445	172	70 1
Total	18	290	54–1870	416	98.0

Table VI. *Blood lipids in patients with ASO. Fredrickson lipoprotein types by different upper normal serum cholesterol limits. Both sexes combined*

Lipoprotein type	Upper normal cholesterol limit: 275 No.	275 (%)	300 No.	300 (%)	325 No.	325 (%)
Normal	23	27.1	32	37.7	42	49.4
Type II	47	55.3	38	44.7	28	32.9
Type IV	11	12.9	11	12.9	11	12.9
Unclassified	4	4.7	4	4.7	4	4.7
Total	85	100.0	85	100.0	85	99.9

age. Furthermore, triglycerides in females with ASO tend to be higher than in the female CHD patients. The relatively small number of patients with ASO warrants great care in drawing conclusions from differences observed in the blood lipid values in patients with ASO and CHD. It should also be remembered that a large proportion of patients with intermittent claudication present symptoms of coronary heart disease as well—36.5% in the Selvaag study (4)—and that CHD is the most common cause of death in patients with arterial insufficiency of the lower limbs (1).

These observations indicate that the underlying basis is the same for both clinical CHD and ASO. Thus measures taken to delay the onset of one should, if successful, delay the onset of the other as well.

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Table VII. *Blood lipids in patients with ASO. Mean values for blood cholesterol, triglycerides, glucose and presence of xanthomas and xanthelasmas in lipoprotein types. Both sexes combined*

	No.	Cholesterol Mean	S.D.	S.E.	Triglycerides Mean	S.D.	S.E.	Glucose Mean	S.D.	S.E.	Xanthoma (No.)	Xanthelasma* (No.)
Normal	23	240	26	5.4	107	43	8.9	81	14	2.9	0	0
Type II	47	340	44	6.4	130	45	6.6	81	19	2.9	3	1
Type IV	11	394	84	25.4	533	476	143.5	79	12	3.7	1	0
Unclassified	4	377	65	32.7	272	107	53.6	79	14	7.2	0	0
Total	85	322	71	7.7	183	220	23.9	81	16	1.8	4	1

* One patient not examined for xanthomas and xanthelasmas.

Table VIII. *Blood lipids in patients with ASO. Relative weight in lipoprotein types. Both sexes combined*

	Underweight (%) >20	Underweight (%) 11–20	Normal weight (%) ±10	Overweight (%) 11–20	21–30	31–40	41–50	Not exam.	Total
Normal	1	4	14	3	1	0	0	0	23
Type II	4	6	22	7	3	3	0	2	47
Type IV	0	0	7	2	1	0	1	0	11
Unclassified	0	0	3	0	1	0	0	0	4
Total	5	10	46	12	6	3	1	2	85
	5.9	11.8	54.2	14.1	7.1	3.5	1.1	2.2	99.9

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THE DIAGNOSIS OF EBSTEIN'S DISEASE OF THE HEART

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Abstract. On the basis of 20 patients from our departments review is given of the clinical, electrocardiographic, radiological and haemodynamic features of Ebsteins disease. The clinical spectrum is very broad, and the presence or absence of cyanosis is essential in the estimation of the severity of the disease, the cyanotic cases usually being diagnosed in childhood. Dyspnoea on exertion, malar flush, palpitations and precordial pains are the most consistent symptoms. The diagnosis is possible on clinical grounds in the typical cases with the combination of systolic and diastolic murmur together with an audible third heart sound, with right bundle branch block, tall P waves, and with considerable enlargement of the right side of the heart on the roentgenogram. In less severe cases right heart catheterization and angiocardiography are needed for confirmation of the diagnosis.

In Ebstein s disease there is a malformation and downward displacement of the tricuspid valve with reduction in size of the functional right ventricular cavity sometimes associated with tricuspid incompetence. Often an atrial septal defect is present also, and in these cases central cyanosis due to a venous-arterial shunt through the defect is usually present.

Since the first description in 1866 (3) several hundred cases have been reported, and the disease was extensively reviewed by Genton and Blount in 1967 (4).

The diagnosis is possible in vivo (13), and in recent years the interest in the disease has been increasing due to reports of successful radical operations during cardiopulmonary bypass (1 6, 7 11)

The purpose of this paper is to point to the characteristic diagnostic features of Ebstein's disease by reviewing 20 cases from our departments at the time of the first admission when the diagnosis was established.

MATERIAL AND METHODS

The 20 patients ranged in age from 2 to 55 years. The distribution according to age and sex is seen in Table I. Phonocardiograms were obtained in all patients. Routine 12-lead ECGs ere obtained in all patients except one, in hom only the standard leads were available. The electrical axis in the frontal plane was determined by measurement of the amplitudes. In all patients the cardiothoracic ratios and heart volumes ere determined. All patients underwent right heart catheterization, including the recording of intracardiac ECGs in tw patients and angiocardiography in 13. The diagnosis was confirmed at autopsy in four patients (cases 2, 7 10 and 16) and at operation in two (cases 1 and 5) The case histories of six patients (cases 3, 7 10, 11 1., 14) have been previously published (5)

RESULTS

Clinical features

The most consistent symptom is dyspnoea on exertion, which was present in all patients but one (case 9) Fourteen patients were in functional capacity class II and the remaining five in class III (Table I). Cyanosis was found in nine patients, eight of whom had clubbing; in one of the cyanotic patients squatting was described (case 1). A malar flush was described in 12 patients, most of whom were cyanotic. In five of the six patients with precordial pains these were related to exertion, one of these patients (case 15) and the remaining patient (case 18) had attacks of pain at rest, lasting for about one half to one hour Two patients (cases 2 and 20) had mild congestive heart failure on admission. Vigorous pulsation of the neck veins was seen in four patients. All patients had a normal blood pressure.

Auscultation

Abnormal auscultatory findings were present in all patients and consisted of either a systolic mur-

Table I *Survey of clinical findings in 20 patients with Ebstein s disease at the time of diagnosis*

Pat. no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Age (y)	2	3	7	8	8	9	10	12	13	18	22	22	25	26	28	28	30	36	43	55
Sex	♀	♂	♀	♀	♀	♀	♀	♂	♂	♂	♂	♂	♀	♂	♂	♀	♀	♂	♂	♂
Functional capacity class	III	III	II	II	III	II	II	II	I	II	II	II	II	II	II	II	II	III	II	III
Dyspnoea on exercise	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+
Palpitations	−	−	−	+	−	−	−	−	−	+	+	−	−	+	+	−	+	−	−	
Precordial pains	−		−	−	−	−	−	+	−	−	+	−	−	+	+	−	−	+	+	−
Cyanosis	+	−	+	+	+	−	+	+	−	+	−	+	−	−	−	+	−	−	−	−
Clubbing	+	−	+	+	+	−	+	+	−	+	−	−	−	−	−	+	−	−	−	−
Malar flush	+	−	+	−	+	+	+	+	−	+	−	+	−	−	+	−	−	+	+	+
Systolic murmur	+	−	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+
Diastolic murmur	−	−	+	−	−	+	−		+	−	−	−	−	+	+	+	−	−	+	+
3rd heart sound	−	+	+		+	+	+	+	+	+	−	+	+	−	+	+	+	+	−	+

mur a diastolic murmur a third heart sound, or a combination (Fig. 1) as shown in Table I. The localization was at the apex and/or at the left sternal border in the third or fourth intercostal space. The systolic murmur was holosystolic of medium to high frequency ranging from grade 1 to 4 (of 6) and was present in 19 patients, two patients had in addition a grade 1–2 systolic ejection murmur in the pulmonary area. The diastolic murmur was usually mid to end-diastolic and of low frequency grade 1 to 2.

Electrocardiography

All patients had an abnormal ECG with bundle branch block; in 18 patients it was right-sided (Fig. 2), in one left-sided (Table II, case 2) and in one with Wolff Parkinson-White block type B it was intermittent right-sided and left-sided (case 12). One patient (case 20) had atrial fibrillation, all others had sinus rhythm. The electrical axis in the frontal plane, the duration and amplitude of the P waves in lead II, the P-Q interval and the QRS duration are shown in Table II. The P waves in lead II were usually broad and tall

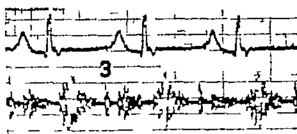


Fig. 1 Phonocardiogram recorded from the fourth intercostal space at the left sternal border in patient 6, showing the third heart sound, the systolic murmur and the diastolic murmur. Paper speed 50 mm/sec. Mingograph 31B, Elema-Schönander

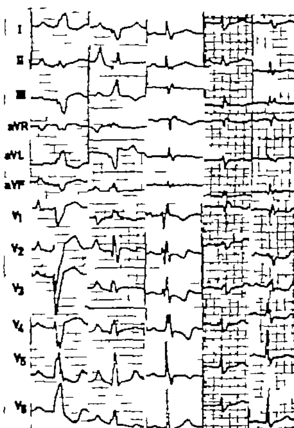


Fig 2. ECGs from patients 2, 3 9 11 and 17 (from left), showing left sided bundle branch block (patient 2, first from left) and fou different types of QRS complexes in right sided bundle branch block. Tall peaked P waves in the standard and precordial leads are seen in patient 3 (second from left) 1 mV = 1 cm (the height of two squares)

and also often prominent in all precordial leads. In eight patients a Q wave was found in lead aVR. The voltage of R and R' in lead V_1 was less than 0.9 mV in 19 patients. A broad S wave in lead V_6 (more than 0.04 sec) was present in 13 patients.





Fig. 3 a and b. Chest roentgenogram, anterior-posterior projection (*a*) and lateral projection (*b*) of patient 8 who was cyanotic. The heart is moderately enlarged especially to the right and less spherical form. The lung vascularity is markedly reduced.





Fig. 4 a and b. Chest roentgenogram, anterior-posterior projection ( ) and lateral projection (*b*) of patient 20, who was acyanotic but with marked symptoms. The heart is huge. The lung vascularity is slightly reduced.

Radiology

All patients had enlarged cardiac silhouettes on the roentgenogram of the chest (Figs. 3 and 4), the cardiothoracic index varying between 49 and 85 (Table II). The right side of the heart was described as enlarged in 11 patients, the left side in two, and both sides in one; in the remaining six cases it could not be determined which cavities were enlarged. The heart volume was usually most increased in the acyanotic patients (Table II).

Table II. *Electrocardiographic and radiologic findings in 20 patients with Ebstein's disease*

Patient 2 had left bundle branch block, patient 12 intermittent left and right bundle branch block and patient 20 *atrial fibrillation*

Pat. no.	1	2	3	4	5	6	7	8	9	10	11	12
Axis frontal plane	+135	−30	+150	−60	+170	−120	−110	+120	+30	−60	−120	−45[a] −10
P_{II} duration (sec)	0.10	0.10	0.08	0.10	0.08	0.10	0.10	0.10	0.08	0.09	0.11	0.10
P_{II} amplitude (mV)	0.25	0.40[a]	0.40	0.40	0.30	0.30	0.40	0.30	0.20	0.40	0.30	0.15[a] 0.25
PQ duration (sec)	0.14	0.22	0.18	0.16	0.17	0.20	0.18	0.17	0.18	0.16	0.24	0.10
QRS duration (sec)	0.10	0.16	0.11	0.11	0.10	0.10	0.10	0.11	0.08	0.14	0.12	0.18[a] 0.12
Heart rate (min)	150	90	90	102	120	86	83	86	73	72	61	58 53
Cardiothoracic index	54	54	74	66	56	57	67	62	52	49	68	59
Heart vol. (ml/m² BSA)	315	635	1 020	780	350	390	750	500	450	580	740	420
Arterial oxygen saturation (%)	68	—	89	84	78	93	87	82	92	89	96	90

[a] When left bundle branch block.

Central lung vascularity was judged to be normal in one cyanotic patient (case 4) and in two acyanotic (cases 6 and 17), and in the remaining patients it was reduced, especially in the cyanotic. The peripheral lung vascularity was decreased in all patients but two (cases 4 and 17), in whom it was described as normal.

Cardiac catheterization

At right heart catheterization an additional atrial septal defect was found in all the nine patients with central cyanosis and decreased arterial oxygen saturation (below 92%) (Table II). A small persistent ductus arteriosus was found in one patient. One patient (case 2) probably had additional pulmonary stenosis, as the pressure in the right ventricle was 56 mmHg in systole and a pulmonary ejection murmur was present, the pulmonary artery was not entered.

In the remaining 19 patients the pressure in the right ventricle was below 30 mmHg in systole; in four of these patients the pulmonary artery was not entered. The mean pulmonary "wedge" pressure was below 7 mmHg in the 11 patients in whom it was measured. The v-wave in the pressure tracing from the right atrium was 13 mmHg and 17 mmHg, respectively in two patients (cases 14 and 20), but in all the remaining 18 patients it was only between 2 and 7 mmHg. The pressure tracing from the right atrium often showed near confluence of the a- and v waves due to a small x-descent.

An intracardiac ECG was recorded in two patients, revealing ventricular QRS complexes from a large area with atrial pressure. The cardiac index was determined in four patients (cases 13, 15, 18 and 20), being 2.6, 1.5, 1.1 and 2.1 l/min/sq.m, respectively.

At selective angiocardiography with contrast injection into the right atrium the diagnosis was confirmed in 12 of 13 patients, as a small right ventricle was demonstrated, situated above and in front of a large right atrium with displacement of the tricuspid ostium to the left. In one patient the angiocardiography was inconclusive.

During catheterization two patients had short lasting episodes of atrial fibrillation and two had short bouts of supraventricular tachycardia, all arrhythmias subsided spontaneously. No serious complications were encountered.

DISCUSSION

Our series confirms the statement made by several authors in recent years (4, 8, 9, 12, 16) that the clinical spectrum in patients with Ebstein's disease is very broad, varying from severely cyanotic cases or cases with congestive heart failure to asymptomatic cases.

The presence or absence of cyanosis is essential in the estimation of the severity of the disease, and the diagnosis in the cyanotic cases is usually established in childhood. The three cyanotic patients in our series, in whom the diagnosis was made in adulthood, were examined shortly after the introduction of the technique of heart

13	14	15	16	17	18	19	20
+120	+120	+30	+150	+120	−30	−30	−120
0.06	0.12	0.11	0.10	0.06	0.10	0.10	—
0.20	0.20	0.40	0.35	0.10	0.20	0.20	—
0.17	0.18	0.20	0.22	0.17	0.16	0.22	—
0.14	0.14	0.12	0.13	0.10	0.16	0.10	0.16
70	58	97	76	50	84	100	75
64	55	69	62	55	51	61	85
475	770	1 200	600	630	600	700	1 700
96	93	98	86	97	97	99	97

catheterization. Our three acyanotic children were referred because of congestive heart failure (case 2) or because a heart murmur was discovered at routine examination (cases 6 and 9).

Dyspnoea on exertion, in cyanotic as well as acyanotic cases, malar flush, palpitations and precordial pains are the most frequent clinical findings.

The combination of systolic and diastolic murmurs together with an audible third heart sound, which is *highly suggestive of Ebstein's disease* (10), was found in 8 of our 20 patients. The murmurs in themselves do not permit any certain diagnosis several of our patients were suspected of having mitral valve disease, probably because the murmurs often were loudest at the apex. Auscultatory findings may be completely within normal limits (7)

The following characteristic *features* of the ECG are stated by Burch and DePasquale (2) 1) paroxysmal atrial or ventricular tachycardia, 2) anomalous atrioventricular conduction, 3) prolongation of the P R interval, 4) tall P waves which are often prolonged in duration, and 5) atypical complete or incomplete right bundle branch block pattern Though no attacks of tachycardia were recorded in our patients, six of them complained of palpitations, and arrhythmias were provoked in four patients during cardiac catheterization. One patient had Wolff Parkinson-White block, which is present in 5–10% of cases with Ebstein's disease and the disease should especially be suspected when the block is of type B (8) The PR (PQ) interval was prolonged in most children in our series. In most of our patients we found tall P waves, but in two patients only the P waves were broader than 0 10 sec. One of our patients had left bundle branch block, which is found in ‒5% of cases (4 14). A Q wave in lead aVR is supposed to be invariably present in Ebstein's disease (2), but we cannot confirm this statement. All cyanotic patients except the one with Wolff Parkinson-White block had P waves in lead II with amplitude equal to or more than 0.25 mV while the amplitude was increased in less than half of the acyanotic patients. None of our patients had an entirely normal ECG but this has been described in infants (8)

The roentgenogram of the chest showed a slight to enormous enlargement of the heart in all patients. In three acyanotic patients (cases 6, 9 and 15) with slight or no symptoms the heart volume was below 500 ml sq.m, while the remaining acyanotic patients with more severe symptoms had volumes of over 600 ml sq.m. Most of the cyanotic patients had heart volumes below 600 ml sq.m. Radiologically there seems to be a distinction between the acyanotic patients with marked symptoms and the cyanotic patients. the former group generally have markedly enlarged *hearts* and only slightly reduced *lung* vascularity while the latter group have less increased heart volumes but more reduced lung vascularity From the chest roentgenogram it might be difficult to determine which heart chamber is enlarged, because of the rotation which the right atrium may cause: this explains why the left side of the heart sometimes erroneously is thought to be enlarged.

At angiocardiography with contrast injection into the right atrium a diagnosis of Fallot's tetralogy is sometimes suggested in the presence of an atrial septal defect, because of the simultaneous filling of both the right and left heart cavities, the often small pulmonary artery and the reduced vascularity of the lungs (10). In these cases the measurement of the pressure in the right ventricle is important and ensures the diagnosis. The recording of an intracardiac ECG may be of help, but both false positive and false negative results may be obtained (8 15). A high mortality has been reported in connection with cardiac catheterization of patients with Ebstein's

disease (10 16) but in our experience the risk is not increased.

The diagnosis of Ebstein's disease is not difficult on clinical grounds in the typical cases, with cyanosis, dyspnoea on exertion, systolic and diastolic murmurs and a third heart sound at the apex or along the left sternal border right bundle branch block with tall P waves, and considerable enlargement of the right side of the heart giving a globe-shaped silhouette on the roentgenogram. But some patients are asymptomatic, some have normal auscultatory findings or a normal ECG and some a normal chest roentgenogram, in these cases the diagnosis can only be suspected clinically and has to be confirmed at cardiac catheterization and angiocardiography

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KIDNEY PRESERVATION BY HYPOTHERMIA USING A PERFUSATE MEDIUM WITH "INTRACELLULAR ION COMPOSITION"

Renal Clearances in Pigs with Autotransplanted 24-hour Preserved Kidneys

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Abstract. Preservation of pig kidneys for 24 hours at 0°C has been studied using combined surface and short-term initial perfusion cooling. As perfusate and storage medium a solution which mimics the intracellular ion composition has been used. Six consecutive experiments were performed. All the animals survived the transplantation and were observed for three months. Measurements of inulin, endogenous creatinine, urea and PAH clearances 10, 31 and 94 days after transplantation showed normal values as compared to a control group of autotransplanted, not long-term preserved pig kidneys. Apart from a transitory reduction in the renal function during the first days after transplantation, the only permanent reduction found was a reduced maximal tubular excretion of PAH.

Renal preservation using hypothermia, performed by means of a short-term perfusion with cold fluid immediately after removal of the kidney followed by surface cooling, is at the present time the most used method of preservation in human transplantation.

The advantages of using this method are obvious. It is easy to perform, can be put to use at a moment's notice, and the risk of technical failures is small as compared to more complicated preservation procedures (12, 13).

The disadvantage has been that only a few hours' storage could be allowed if immediate and fully reversible kidney function of the transplanted kidney were to be obtained. In experiments with dogs the use of blood (2, 8, 16), serum (19), isotonic NaCl (18) and low molecular weight dextran in balanced salt solution (TIS-U SOL, Rheomacrodex® c NaCl) either alone (1, 9) or followed by perfusion with equal parts of 1.3% $NaHCO_3$ and 10% invert sugar (3) have allowed preservation for 20–24 hours only if the contralateral nephrectomy was postponed for 2–5 weeks. The kidney function in the surviving animals mostly showed some degree of permanent reduction.

A perfusate medium which mimics the intracellular ion composition has recently been described to have a better effect of preservation than the perfusate media mentioned (4). Hypothermic storage for 30 hours with this fluid revealed only a moderate and transitory increase in serum creatinine after transplantation, even if the contralateral nephrectomy was carried out in connection with the transplantation.

This considerable improvement has, however, been questioned in preliminary reports (5, 17), and it is therefore the aim of the present study to describe our experiences with this solution, and to compare the method with preservation experiments earlier published by us (7).

MATERIAL AND METHODS

Six female pigs of the Danish Landrace breed, 4 to 5 months of age, and weighing from 37 to 53 kg at the time of surgery, were used for the study. During the first 1–3 days after transplantation the animals were kept on a protein-restricted diet, to which 5 g $NaHCO_3$ was added. Water was restricted until abundant urine production was observed (0–2 days after transplantation). During the remaining experimental period the pigs were fed with a standard fodder mixture (6) and unlimited quantities

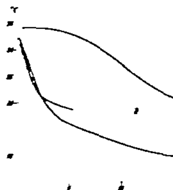


Fig 1 Cooling curves from the core of pig kidneys. *A*, Surface cooling (0°C at the surface). *B* Ten min perfusion with 200-300 ml cooled perfusate (5°C). *C* Combination of surface and perfusion cooling. The distance from the thermistor to the nearest point at the surface as about 1.5 cm. T experiments in each group.

of water were permitted. The animals were weighed once week, and the daily administration of fodder was calculated on the basis of the body weight.

Preservation of the kidney

All the autotransplanted kidneys were cooled with combination of perfusion and surface cooling and stored for 4 hours at 0°C.

The perfusate medium was nearly similar to that described by Collins et al. (4) as the "C"-solution using a basis solution (containing $K_2HPO_4 3H_2O$, 9 7 g, KH_2PO_4 ...05 g, KCl, 1.. g, $NaHCO_3$, 0.84 g and aqua sterilisata ad 1 000 ml) to which papaverine (50 mg l), glucose (20 g l) and $MgSO_4$ (7.38 g l) were added immediately before use.

About half an hour before removal of the kidney 500 ml 10% mannitol was given intravenously together with 1 000 ml isotonic NaCl. Five minutes before, the animals were heparinized (5 000 I.U heparin 10 kg b.wt.). As soon as possible after clamping of the renal artery (1-3 min), the kidneys were placed in crushed glucose ice (isotonic glucose) and perfused with 200-300 ml cooled perfusate (5°C), using pressure from 100 to 150 cm H_2O. In tw cases the temperature was measured in the core of the kidney during the perfusion by means of thermistor (Yellow Springs type 5?4).

The kidneys were stored at 0°C in solution similar to the perfusate medium, with the only exception that magnesium sulphate was omitted to prevent precipitation of heavily soluble magnesium phosphates in the storage medium.

After 4 hours storage the kidneys were reimplanted as described below. During surgery the kidneys were wrapped in cooled surgical dressings.

Surgical technique

Due to series of surgical complications, especially in the form of postoperativ arterial thrombosis and mechanical ileus, we hav changed the surgical technique described earlier (7). Nephrectomy as well as renal autotransplantation were performed with an extraperitoneal technique and the preserved kidney was placed at the site of the opposite and just removed kidney using end-to-end anastomosis between the renal vessels. A uretero-ureteral anastomosis was performed. Details are described elsewhere (15).

Preoperative studies

Thirty minutes after recirculation, renal blood flow as measured by means of the Xenon-133 wash-out technique. 0.1 mCi was injected into the artery and the disappearance of the isotope was followed with an external scintillation detector (11.5 × 11.5 mm NaI crystal) placed directly over the kidney. The detector was coupled to rate meter and to semilogarithmic recorder (Medtronic). The blood flow was calculated according to the initial slope method (10). The blood pressure was measured by puncture of the aorta and registered by means of pressure transducer (Statham type P 23AA).

One hour after recirculation biopsy was taken from the kidney

Postoperative studies

Blood analyses. During the first week after the surgery blood samples were taken every other day and subsequently once week for the following three months. The pH and haematocrit values were determined, together with the concentrations in plasma of creatinine, urea, sodium and potassium.

Kidney function. The clearances of inulin, endogenous creatinine, urea, para-aminohippuric acid (PAH), and the excretion percentages of water sodium, potassium and chloride were determined. These measurements were performed on unanaesthetized animals 10, 31 and 94 days after transplantation. Each experiment comprised at least three periods of 20 min, and the last experiment was concluded with three periods of 20 min to determine the maximal tubular excretion (Tm) for PAH. Three days after the last clearance experiments the extraction percentage for PAH as determined, after which the animals were killed. Details of the doses of test substances, the technique and the calculation methods hav been published previously (6).

Analytical methods have been described previously (6).

Postmortem examinations. After the observation period the animals were killed and bled, and postmortem examination was performed. The kidneys were eighed.

Histological examinations. At necropsy kidney tissue was removed and fixed in neutral buffered formalin. The biopsies taken one hour after recirculation were fixed in Zenker' fluid and in neutral buffered formalin. Paraffin wax sections were stained with iron haematoxylin-van Gieson, and the periodic acid Schiff reaction was carried out according to McManus and Mowry (14).

RESULTS

The perfusion

Fig. 1 shows the rate of cooling in the kidneys (weight about 150 g, size 13 × 6 × 3 cm) during the first 15 min after removal (curve C). A com-

Table I. Renal blood flow in 24-hour preserved autotransplanted pig kidneys 30 min after recirculation compared with blood flow in non-transplanted pig kidneys. Determined by means of Xenon-133 wash-out technique

24-hour preserved kidneys		Non-transplanted kidneys	
Pig no.	Flow (ml/100 g/min)	Pig no.	Flow (ml/100 g/min)
123	—	92	266
124	140	93	73
125	95	94	11
126	112	95	246
127	210	96	120
128	59	99	103
Mean 123 ± 57 (S.D.)		Mean 153 ± 81 (S.D.)	

$0.50 < p < 0.60$

parison with the rate of cooling when using only surface (curve A) or perfusion (curve B) cooling has been made

Initial behavior

After removal from the storage chamber an average of 35 min passed before recirculation was established. Immediately after recirculation all the kidneys turned pink and became normal in consistency. This remained unchanged during the surgery. The urine production started within a few minutes and continued during the surgery.

The blood flows of the preserved kidneys 30 min after recirculation are shown in Table I together with blood flow determinations in non-transplanted pig kidneys, measured with the same technique. It can be seen that the dispersions in both groups are relatively great and that no signif



Fig. 2. (A) Histological section of kidney biopsy from pig 127 one hour after recirculation. Normal proximal tubule with distinct brush border (*a*). Hyaline PAS positive droplets in the cytoplasm and tubular lumen (→). Zenker fluid PAS reaction. ×190.



(B) Histological section of the kidney from pig 126 three months after transplantation. Slight interstitial fibrosis (*b*), but otherwise normal. Buffered formalin. Iron haematoxylin–van Gieson. ×75.

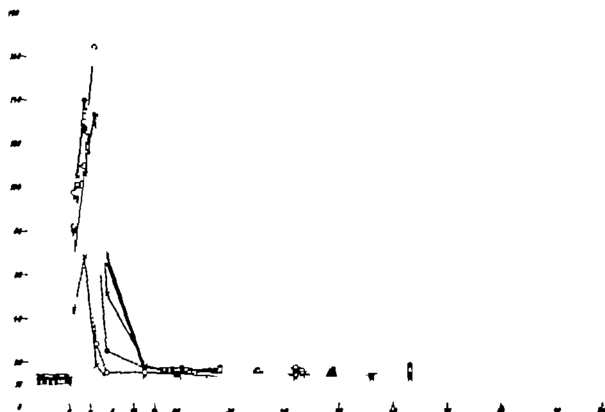


Fig 3 Concentration of creatinine in plasma 0–94 days after transplantation. Ordinate: creatinine in plasma, μg/ml. Abscissa, days after transplantation. ●—● pig 123 ○—○ pig 124 ×— pig 125 ● ● pig 126; ○--○ pig 127 -- pig 128

icant difference was found The blood pressure was found to be within normal limits in all cases.

The histological picture one hour after recirculation is shown in Fig 2 A. In the proximal tubules varying degrees of cellular desquamation, hyaline droplets and hyaline casts were found. The remaining structures of the kidney had a normal appearance.

Subsequent function

All the animals survived the transplantation and were followed for three months.

Concentrations of creatinine, urea, sodium and potassium in plasma Figs 3 and 4 show the concentrations of creatinine and urea in plasma (μg/ml), respectively The concentrations of sodium and potassium were constant throughout the period, 147 ± 4.3 (S D) and 5 1 ± 1.5 (S.D), respectively The pH remained constant, averaging 7 41 ± 0.05 (S.D).

Clearances of inulin, endogenous creatinine urea and PAH Table II gives the average values for clearances of inulin, endogenous creatinine, urea and PAH, for the effective renal plasma flow ($RPF_{effective}$) and effective renal blood flow ($RBF_{effective}$) The clearance ratios are stated. Since the animals were killed immediately after the last clearance experiment, it was possible to calculate the clearance both per 10 kg b.wt. and per 100 g kidney tissue. The results are given in Table III, which also shows the extraction percentage for PAH used for calculating the total renal blood flow (RBF_{total}).

Maximal tubular excretion of PAH In the experiment 94 days after transplantation the Tm of PAH was determined, and the results for each individual pig are shown in Table IV which also shows the concentrations of PAH in plasma at which the Tm was determined. The Tm values were calculated both per 10 kg b.wt. and per 100

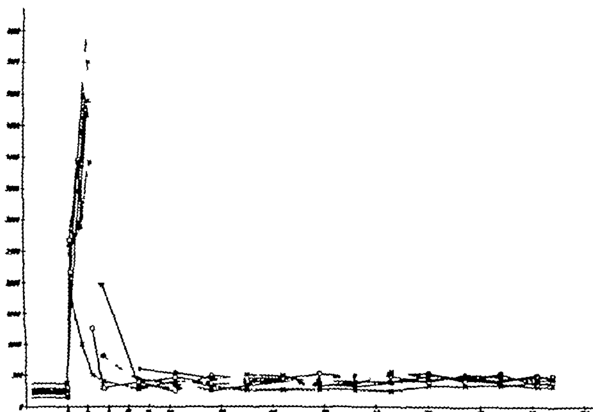


Fig. 4 Concentration of urea in plasma, 0-84 days after transplantation. Ordinate: urea in plasma, µg/ml. Abscissa: days after transplantation. ●—● pig 123 ○—○ pig 124 — pig 125 ● ● pig 126; ○- -○, pig 127 pig 128.

g kidney tissue, and are on average 16 mg/min/10 kg b.wt. and 49 mg/min/100 g kidney tissue.

Excretion of water sodium, potassium and chloride The average excretion percentages for water sodium, potassium and chloride are shown in Table V The average values for the whole period were 1.57 ± 0.45 (S.D.), 0.32 ± 0.28 (S.D.), 31 ± 13 (S.D.) and 0.95 ± 0.58 (S.D.) respectively

Postmortem examination

Macroscopical findings All the kidneys were normal in colour and consistency Cortex and medulla had a normal appearance. The kidneys from pigs 123 1.5 and 126 were difficult, the others easy to decapsulate. Vascular and ureteral anastomosis were without complications.

Microscopical findings. In the kidneys from pigs 124, 125 and 127 a slight dilation of the glomerular spaces and the tubules was seen. Focal infiltration with histiocytes was seen in the kidney from pig 128. Otherwise the kidneys showed no pathological changes, and especially no or only minimal interstitial fibrosis could be found (Fig. 2 B).

DISCUSSION

Cooling of kidneys after removal from donors can be carried out by means of surface cooling, perfusion with cold fluid or by a combination of both methods.

To decrease the metabolism as fast and as much as possible, we have chosen a combined surface and perfusion cooling and a storage temperature at 0 C. With this technique the temperature decreased below 10 C in about 15 min in the deepest parts of the kidney (Fig. 1). In our experiments this method did not reveal any non reversible damage to the kidneys, which is in agreement with others (2, 4).

Our perfusate medium and perfusion technique

Table II. *Average renal clearances in six pigs 10–94 days after transplantation*

The figures in parentheses indicate minimal and maximal values of single experiments

				Clearance			
D after transplantation	B.wt. (kg)	Haematocrit (%)	Diuresis (ml/min)	Inulin (ml/min/10 kg b.wt.)	Endogenous creatinine (ml/min/10 kg b.wt.)	Urea (ml/min/10 kg b.wt.)	PAH (ml/min/10 kg b.wt.)
10	54 (44–65)	31 ([illegible]4–35)	1.7 (0.9–[illegible]9)	16 (1[illegible]–19)	16 (14–19)	9 (6–11)	57 (51–69)
31	68 (60–86)	38 (34–41)	1.9 (1.[illegible]–[illegible]5)	19 (16–21)	[illegible] (20–[illegible]1)	11 (10–13)	72 (52–88)
94	110 (99–126)	41 (36–45)	2.4 (1.4–3.7)	19 (17–[illegible]2)	19 (17–21)	11 (9–12)	69 (50–81)

differs only little from that described by Collins et al. (4). We have added papaverine to the perfusate medium instead of phenoxy-benzamine and procaine, because earlier studies have revealed a reliable vasodilatator effect of papaverine in hypothermic kidney perfusions (11). With the concentrations used we have had no problems with the solubility of papaverine in the perfusate medium, in contrast to the experiences of Collins et al. with phenoxybenzamine (4). The addition of magnesium sulphate in the concentration used revealed constant macroscopical precipitation after some hours (heavy soluble magnesium phosphates), which necessitated addition of this component immediately before use.

Blood flow determinations by means of the Xe 133 wash-out technique 30 min after recirculation showed great variations in preserved as well as non-transplanted kidneys. The blood flow in normal pig kidneys is 364 (300–440) ml/min/100 g kidney tissue, using PAH clearance determination (6), whereas the average blood flow was 153 (73–[illegible]66) ml/min/100 g kidney tissue when the Xe-133 wash-out technique was used during the surgery in normal pigs. The lower values obtained by the Xe-133 wash-out technique are probably due to vasospasm in the kidneys in connection with the surgical manipulations.

The concentrations of creatinine and urea in plasma, as well as different renal clearances, were compared with results of a control group of autotransplanted, not long-term preserved kidneys, which have been published previously (7 group C).

The concentration of creatinine and urea in plasma increased during the first days after transplantation (Figs. 3 and 4), fell after the fifth day and were stabilized within 10 days. During the period from 10 to 94 days after transplantation the average concentration of creatinine in plasma was 15 $\mu g/ml \pm 0.8$ (S.D.), while it was 14 $\mu g \pm 0.5$ (S.D.) in the control group ($0.10 < p < 0.20$). Compared with the control group the concentra

Table III. *Renal clearances and total renal blood flow in six pigs 94 days after transplantation*

	Clearance										
	Inulin		Endogenous creatinine		Urea		PAH			Total renal blood flow	
Pig no.	ml/min/10 kg b.wt.	ml/min/100 g kidney	ml/min/10 kg b.wt.	ml/min/100 g kidney	ml/min/10 kg b.wt.	ml/min/100 g kidney	ml/min/10 kg b.wt.	ml/min/100 g kidney	Extraction of PAH (%)	ml/min/10 kg b.wt.	ml/min/100 g kidney
123	17	56	17	56	12	40	71	[illegible]34	89	135	446
124	20	70	19	67	11	39	77	270	85	156	548
125	18	52	20	58	9	26	71	208	87	151	440
126	17	56	18	60	10	33	50	166	88	111	372
127	19	53	21	59	9	25	81	228	85	188	530
128	22	57	18	47	12	31	66	172	81	159	412

...ome ...al ...ma flow ...l/min/10 ...)	Effective renal blood flow (ml/min, 10 kg b. t.)	Clearance ratios		Filtration fraction In/PAH
		Cr/In	Urea/I	
(55-75)	91 (79–115)	1.0	0.6	0 28
(87-98)	127 (90–160)	1 1	0.6	0.26
(54-88)	129 (98–60)	1.0	0.6	0.28

tion of creatinine and urea in plasma during the first days after transplantation were somewhat higher but from about 7 days after transplantation no difference could be found.

The inulin, endogenous creatinine, urea and PAH clearances on the 10th, 31st and 94th day after transplantation showed no significant dif ference (on the 0 01 level) compared with the control group. On the 94th day the average values were 19 19 11 and 69 ml/min/10 kg b.wt., re spectively while the values in the control group were 18 18 10 and 69 ml/min/10 kg b.wt.

The excretion percentages of sodium, potassium and chloride were almost identical to values obtained in the control group. The Tm of PAH was on average 16 mg/min/10 kg b wt. ± 3.8 (S D), and in the control group 25 mg/min/10 kg b.wt. ± 4 4 (S.D), ($p < 0.001$) While the preservation had no influence on the filtration and PAH clearances, nor on the blood flow of the kidney the estimation of the Tm of PAH shows that the preservation has lowered this parameter The ex traction percentage of PAH was 86 ± 3 (S.D) which is near the values in normal pigs (6)

The average ratio between endogenous creatinine and inulin clearance was 1.05 ± 0 09 (S.D.) while it was 1 09 ± 0 14 (S D) in the control group ($0.7 < p < 0.8$)

The average filtration fraction was 0.27 ± 0.04 (S.D) against 0.29 ± 0.05 (S.D) in the control group ($0.05 < p < 0.10$).

Our results confirm the results obtained by Collins et al. (4) and support the assumption that the perfusate medium used has a significantly better preservation effect than the perfusate media mentioned in the introduction. Compared with our earlier experiences with hypothermia and hyper baric oxygen (12) and hypothermia serum perfusion (13), the present method revealed the best results, judged by a quick return to normal func tion after transplantation of the preserved kidney and with consistently good results in a consecutive series.

Table IV *Clearance and Tm f PAH 94 days after transplantation*

Pig no.	B wt. (kg)	Kidney weight (g)	Relative kidney weight (‰)	Plasma concentration of PAH (µg/ ml)	PAH clearance (ml/min/10 kg b t)	Inulin clearance (ml/min/10 kg b.wt.)	Tm (mg/min/10 kg/b t.)	Tm (mg/min/100 g kidney)
123	104	315	0.30	1 160	25	14	12	38
124	102	291	0.29	1 300	27	17	12	41
125	99	340	0.34	1 440	31	17	20	59
126	108	325	0.30	1 350	27	12	19	63
127	118	420	0.36	1 200	27	10	21	59
128	128	490	0.38	1 000	26	12	14	37

Table V *Average renal xcretion f water and lectrolytes in six pigs 10–94 days after transplantation*

D after transplantation	B.wt. (kg)	Diuresis (ml/min)	Inulin clearance (ml/min/10 kg b.wt.)	Excretion (%) Water	Sodium	Potassium	Chloride
10	54	1 7	16	2.0	0.35	26.8	0.97
31	68	1.9	19	1.5	0.34	34.2	1 19
94	110	2.4	19	1.2	0.26	20 0	0.71

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Acta med. scand. Vol. 189 pp 529–535 1971

THE DIAGNOSIS OF HEPATOBILIARY DISEASES BY SERUM ENZYME ANALYSES

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Abstract. Serum guanase has been observed to rise sensitively in hepatitis but was quite insensitive indicator of extrahepatic jaundice and liver cirrhosis. Thus serum guanase determination, despite its relative specificity for liver damage, appears not to be sensitive enough for an indicator test of liver disease. In respect to sensitivity the serum γ-glutamyl transpeptidase (γ-GT) test was observed to fulfil best the expectations also in anicteric patients, and was elevated particularly in extrahepatic biliary obstruction. Serum ornithine carbamoyl transferase (OCT) activity was observed to rise in hepatitis and in extrahepatic jaundice. Because of its specificity for liver tissue, OCT is the most certain test for detection of liver damage. The differentiation of hepatocellular jaundice from extrahepatic obstruction was noted to be best achieved by means of the serum guanase and alkaline phosphatase ratio. This still leads in about 10% to misleading diagnosis in the differentiation of these clinical states.

The diagnostic serum enzyme tests aiming at the disclosure of liver disease should fulfil several requirements. Firstly they should be sensitive enough to reveal the liver damage also in anicteric patients. Secondly they should, in an ideal form, be specific, and thirdly they are expected to differentiate hepatocellular jaundice from extrahepatic obstruction. A great number of enzymes, abundant in liver tissue, have been tested for their diagnostic capacity beside the conventional tests of aspartate amino transferase (GOT), alanine amino transferase (GPT) and alkaline phosphatase (APh). Lately several new enzyme tests have been introduced in the hope that they would ameliorate the specificity and help in the decision between hepatocellular and extrahepatic jaundice. Of these tests, the determination of ornithine carbamoyl transferase (OCT), which fulfils high qualifications of specificity (33, 34), has been simplified (17 18). Guanase has been observed in animal experiments to be elevated in hepatocellular injury caused by carbon tetrachloride but not after the ligation of the common bile duct (12). And γ-glutamyl transpeptidase (γ-GT), reported to be abnormalized particularly in biliary obstruction (2, 24 35 37) and in liver tumours without jaundice (16 36) might be expected to improve the detection of hepatobiliary disease.

MATERIAL AND METHODS

The 105 patients were grouped into four main groups: (*a*) hepatitis, 32 patients; (*b*) biliary obstruction by tumours or metastases, 20 patients, ( ) extrahepatic benign biliary obstruction, 29 patients, and (*d*) cirrhosis of the liver, 24 patients.

In the hepatitis group (group (*a*)) 12 patients were considered to suffer from acute inoculation hepatitis. Most of them had history of continuous self-administration of narcotics. Acute infectious hepatitis was the diagnosis in 16 patients. All these 28 patients had prompt rise and abnormalization of serum GOT and GPT activities, and the liver biopsy showed histological changes supporting the diagnosis of acute viral hepatitis. In 1 patient thioridazine was the most probable cause of toxic hepatitis. In 3 patients the histological picture obtained from samples taken at laparotomy revealed subacute cholangiolitic hepatitis.

The biliary obstruction was confirmed by laparotomy or autopsy and by operative cholangiography and by mechanical sounding of the biliary passage in groups (*b*) and (*c*). In the tumour group cancer of the head of the pancreas was the cause of the obstruction in 10 patients; 5 had primary liver cancer, 3, cancer of the gall bladder and 2, cancer of the stomach with metastases. In these patients hepatic metastases were sought at operation and additionally by liver scanning by means of

Table I. *Mean serum enzyme activities with standard errors in 105 patients with hepatobiliary disease of various etiologies*

Numbers in parentheses show pathological values. The total series consists of 79 icteric and 26 anicteric patients

Diagnosis	No of pats.	Guanase	γ-GT	OCT	GPT	GOT	APh
Hepatitis	32	12.9 ± 1 7 (29)	71.5 ± 19 1 (26)[a]	1.57 ± 0.17 (31)	360.5 ± 75 0 (30)[b]	263.6 ± 45 9 (30)	72.6 ± 5 7 (27)
Tumour obstruction	20	4 4 ± 0 8 (8)	174.0 ± 30.7 (19)	0.91 ± 0.17 (17)	53.2 ± 11.0 (16)[a]	63.0 ± 13.4 (16)	179.4 ± 25.6 (20)
Non-tumorous obstruction	29	4.5 ± 0.7 (13)	136 0 ± 24.3 (25)	1.01 ± 0.13 (24)[a]	102.0 ± 16.6 (26)	57 7 ± 10.3 (23)	104.9 ± 9.3 (26)
Cirrhosis of the liver	24	2.9 ± 0.5 (10)	132.5 ± 27 9 (23)	0 49 ± 0.10 (11)[a]	34.6 ± 6.8 (14)	39 5 ± 5.8 (19)	58.3 ± 8 7 (12)
Share of pathological values ( )		57	90	81	84	84	81

[a] Determination failed in 1 patient.
[b] Determination failed in 2 patients.

...an colloid. In 8 patients the primary tumour or metastases were found with certainty within the liver parenchyma.

In group ( ) the extrahepatic obstruction in 16 patients was observed to be stones in the common bile duct, in 4 benign stenosis of the sphincter of Oddi; in 1 *sclerosis of the choledochus in 1 gangrenous* cholecystitis, and in 1 benign cystadenoma of the pancreas. In 3 patients pancreatitis was associated with stones in the gall bladder In 3 patient there were findings of stones in the gall bladder and chronic cholecystitis, but at operation no calculi were detected in the common bile duct.

In the patients with liver cirrhosis (group (*d*)) the diagnosis depended on the histological picture of the biopsy material in all except 1 patient. In this case intermittent icteric periods had followed heavy drinking bouts. He had palmar erythema, cutaneous spiders and esophageal varices, and the prothrombin time did not respond to intramuscular vitamin administration. Heavy consumption of ethanol was considered to be the etiology of the cirrhosis in 17 patients. The cirrhosis was evidently posthepatic in 4 patients, in 1 patient it was either post-necrotic or biliary while in 2 patients no certain cause could be found for the cirrhotic changes in the liver In 2 alcoholic patients the hematologic parameters and serum lipid analyses as well as the serum LDH isoenzymes corresponded to the Zieves syndrome.

Serum samples were taken in the acute phase of disease except in 3 patient with stones in the gall bladder and 1 patient who, in addition, had pancreatitis. These patients were studied about 2 weeks after the acute phase of disease. In order to make the serum enzyme activities comparable with each other the unhemolyzed sera were taken simultaneously for all enzyme determinations. OCT (18) and guanase (19) were measured as described earlier from our laboratory The advantage of these tests is their simplicity and, in addition, in the *case of guanase*, the improved sensitivity of the method.

Serum γ-GT was measured according to Kulhánek and Dimov (21). Serum GOT (13), GPT (38), APh (4), as well as serum bilirubin, protein electrophoresis, prothrombin time, serum and urine amylase and urine bilirubin, urobilin and urobilinogen determinations were made according to the routine methods.

Normal values for the serum enzyme analyses were calculated from the determinations of 30 healthy subjects The 99% confidence limit ($p < 0.01$) was used for the upper normal limits. These were as follows: OCT = 0.4 IU/l, guanase = 3.5 IU/l, γ-GT = 20 IU/l, GOT = 18 IU/l, GPT = 16 IU/l, and APh 46 IU/l.

RESULTS

In the total series of 105 patients the serum OCT GOT GPT and APh activities were quite similarly abnormal (in 81–84%), if only the number of abnormal values is considered, whereas serum guanase was abnormal in only 57% and γ-GT in 90% in the total series (Table I). When the degree of abnormalization was considered as an index of sensitivity serum γ-GT and GPT were the most sensitive indicators, but considerable differences were observable according to the etiology of the liver disease (Figs. 1 and 2).

Serum guanase activity was elevated particularly in patients with hepatitis, but was normal or less elevated than the other serum enzymes in extrahepatic biliary obstruction (Figs. 1 and 2) Serum GPT showed a similar feature, but the difference between hepatitis and obstruction was not clear since in calculous obstruction the serum GPT often had risen to high values. The opposite rela-

tion was seen most clearly in serum APh (Fig. _) and γ-GT activities (Fig. 1).

In order to find the best method to distinguish hepatocellular jaundice from extrahepatic obstruction, the simultaneous determination of serum guanase and APh or γ-GT seems to be the most promising. If only the jaundiced patients with hepatocellular damage and patients with biliary obstruction are considered, the best differentiation of these clinical states was achieved by determination of serum guanase and APh.

In Fig. 3 the activity of serum guanase is compared with APh in the 60 patients confirmed to have jaundice due to hepatocellular (31 patients with hepatitis) or biliary obstruction (14 patients with choledocholithiasis and 15 patients with tumour obstruction). To obtain better information on the degree of elevated activities, the relative values are used. Unity is the upper normal limit and the numbers express how many times this is exceeded. The hepatocellular index is characterized by a greater rise of guanase and the obstructive index by a greater rise of APh than of guanase. False information was received in 7 out of 60 cases (1_%). When guanase activity was compared with the activity of serum

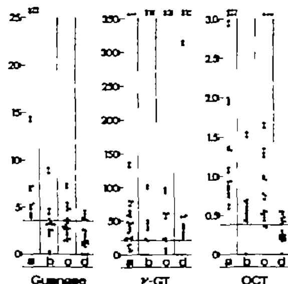


Fig. 1. Serum guanase, γ-GT and OCT activities in 32 patients with hepatitis (*a*), in 20 with tumour obstruction (*b*), in 29 with benign extrahepatic obstruction (*c*), and in 24 with liver cirrhosis (*d*). Enzyme activities are in IU/l. The upper normal limit with 99% confidence is marked by horizontal lines.

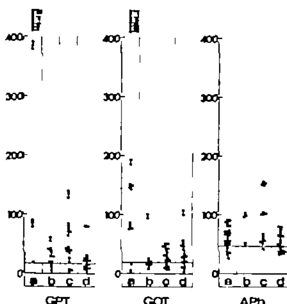


Fig. Serum GPT, GOT and APh in the same patients as in Fig. 1.

γ-GT the information was misleading in 13 patients (Fig. 4). When the patients (20 patients with hepatitis, and 18 with extrahepatic obstruction) with the most marked hyperbilirubinemia (>5 mg/100 ml) were considered, the serum guanase APh ratio had been given misleading diagnosis in 3 patients and the guanase -GT ratio in 6 patients. The other test combinations which reflect a hepatocellular or an obstructive state are presented in Table II.

Serum γ-GT activity has been reported to rise sensitively not only in cases of biliary obstruction but also of hepatic tumours or metastases. In the present study the serum γ-GT activity was observed to rise most in biliary obstruction and less in hepatitis, although it was abnormal in hepatitis in all except 5 cases. The degree of abnormalization was, however, considerably less in hepatitis than in extrahepatic obstruction (Fig. 1). In the 8 patients who had a tumour or metastases within the liver the serum γ-GT did not exceed the activities encountered in other liver diseases, and it seems to fall to the lower range of the activities observed in extrahepatic biliary obstruction. In 5 patients the activity was only moderately elevated (less than 80 IU/l) and in a further 3 patients it was 147, 15_, and 300 IU/l. In the 4 patients who also had associated pancreatitis,

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Table II. *The capacity of various enzyme test ratios to differentiate hepatocellular damage from extrahepatic biliary obstruction. Column A shows how often a correct diagnosis was obtained in 60 icteric patients, while column B shows 38 patients who had hyperbilirubinemia of more than 5 mg/100 ml*

Enzyme combination	A Correct diagnosis (%)	B Correct diagnosis (%)
Guanase/APh	88	92
Guanase/γ-GT	80	84
GOT/APh	77	74
GOT/γ-GT	83	87
GPT/APh	71	71
GPT/γ-GT	77	77

thiasis, however the serum bilirubin was normal in 3 cases and only slightly elevated in one, 1.9 mg/100 ml).

Serum γ-GT as well as the other enzymes were not in correlation with serum bilirubin but, in general, were more clearly elevated in the patients who had hyperbilirubinemia. In the patients who were not jaundiced and whose serum bilirubin was within normal limits the serum γ-GT activity was more distinctly and somewhat more often pathological (in 22 out of 26 patients) than the other serum enzymes (Table III).

DISCUSSION

In recent years clinical interest has been directed to guanase because, outside of the liver this enzyme has been found in human tissues in considerable activity in the kidney and the brain only (15 23). Thus the determination of serum guanase activity might be expected to offer an improved specificity over serum GOT or GPT determinations in the confirmation of liver disease. The clinical value of the serum guanase determination is not, however established, but research has advanced to the introduction of methods for clinical use (6, 12, 19 23). In connection with these methodological studies serum guanase has been observed to reflect hepatocellular damage. In animal experiments serum guanase has been reported to rise in chemical hepatitis but not after surgical ligation of the common bile duct (5 12). In a study of 28 patients, most of them with cirrhosis or fatty changes of the liver serum guanase was noted to abnormalize much less sensitively than serum GOT GPT or APh (25).

In the present series the serum guanase activity was pathological in 29 out of 32 patients with hepatitis. On the other hand, in the patients with extrahepatic obstruction and liver cirrhosis the serum guanase activity was pathological in only 41% of the cases. Thus serum guanase seems to abnormalize sensitively in hepatitis but is not sensitive enough for an indicator test of liver disease, because it does not reflect sensitively the other liver diseases. In anicteric patients serum guanase was observed to be pathological in about a third of the patients in whom serum OCT GPT or γ-GT was abnormal.

By means of the serum OCT test the liver damage can be confirmed more specifically (32, 33 34) than by serum GOT or GPT tests, since OCT is found outside the liver in the small intestine only where its activity is 14% of that found in the liver (31). This great specificity for liver tissue may be of conclusive significance if simultaneous injury in several organs is suspected. For instance, in alcoholics lesions have been observed in skeletal muscles (8 9 10, 14 29 30). Myopathic lesions also seem to be not uncommon, as supported by the elevated serum creatine kinase activity in about 40% of alcoholics (27). Skeletal muscle injury can be revealed

Table III. *The number of pathological serum enzyme activities in 26 anicteric patients with various hepatobiliary diseases*

Diagnosis	No of pats.	Guanase	γ-GT	OCT	GPT	GOT	APh
Hepatitis	1	1	1	1	1	0	0
Tumour obstruction	6	0	6	3	4	1	6
Non-tumorous obstruction	14	4	10	11	11	8	10
Cirrhosis of the liver	5	2	5	3	3	4	1
No. of pathological values		7	22	18	19	13	17

by serum creatine kinase determination in alcoholics with liver damage, since this enzyme is not found in the liver tissue (7). So in an earlier study (?0) on 100 alcoholics it seemed evident in the light of multiple serum enzyme analyses that the serum GOT was in part derived from skeletal muscles, where GOT activity is close to that found in liver tissue (3).

In the 17 cirrhotic patients of the present series who were alcoholics the serum GOT activity was pathological more often (in 15 patients) than were the more specific liver damage indicators. Serum OCT and also GPT were pathological in 9 of these patients, and alkaline phosphatase in 8 The possibility naturally remains that serum OCT does not rise so sensitively as GOT in cirrhotic lesions. This possibility is difficult to explain, however since the opposite relation was seen in hepatitis and extrahepatic biliary obstruction. It may be concluded that serum OCT is a sensitive indicator test for liver disease, and its great specificity prevents a misleading interpretation in patients who might have simultaneous lesions outside the liver

Serum γ-GT has been reported to rise particularly in patients with biliary obstruction (2, 16, 24 35 36, 37), which this enzyme test appears to reflect more sensitively than APh (24) as was confirmed also in the present series. Elevated serum γ-GT activity has been considered to be a consequence of impaired excretion of the enzyme via the bile ducts into the intestine (35) in a similar manner to APh. In addition serum γ-GT seems to abnormalize also in hepatocellular damage, although to a lesser degree than in obstruction. Also in anicteric patients the serum γ-GT was most often pathological and in cirrhotic patients its abnormalization exceeded that of the other enzymes.

Serum γ-GT has been reported to have a prolonged elevation after acute myocardial infarction (1 11). This has recently been interpreted to be a reflection of reparative processes in the myocardium, although its activity in the myocardium is negligible (31). It may be questioned whether a similar reparative process in the liver would be responsible for the serum γ-GT being more often elevated in cirrhosis than in hepatitis the opposite might be expected since the serum bilirubin was more elevated in patients with hepatitis.

In studying an icteric patient an important challange for a clinician is often whether the jaundice is of extrahepatic or parenchymatous origin. Intrahepatic cholestasis, encountered in hepatocellular damage, may cause difficulty in the interpretation of the results. By combining tests of opposite character reflecting either obstructive or parenchymatous jaundice, it is possible to reach some improved diagnostic certainty The most useful tool for this differentation has been the combined determination of serum GOT (or GPT) with APh (22). Such a combination still leaves much to be hoped for in accuracy and efforts have been made to improve it by the simultaneous determination of serum GPT and γ-GT activities (2).

In the present series the combined determination of serum guanase with APh was observed to give the best differentiation of hepatocellular damage from extrahepatic obstruction, and it exceeded somewhat in accuracy all other test combinations. However this test combination still gives misleading information in about 10% and may furthermore often not be of distinctive significance in this differentiation. Serum enzyme determinations in jaundiced patients must therefore be supplemented by other liver function tests, liver biopsy liver scanning, angiography cholangiography laparoscopy and laparotomy

ACKNOWLEDGEMENT

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Acta med. scand. Vol. 189 537–539 1971

SURVIVAL TIME IN CARDIOGENIC SHOCK

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Abstract. Thirty consecutive patients with an acute myocardial infarction and cardiogenic shock have been classified at autopsy as having or not having right ventricular hypertrophy (RVH). The RVH group had significantly longer survival time after onset of shock than those without RVH. The finding is discussed.

The pathophysiology of shock complicating acute myocardial infarction is still not well understood. The stroke volume is reduced but often not more than as is seen in, e.g., severe mitral stenosis. Therefore at least one more factor must be operating. One may be the *sudden* reduction of the stroke volume in acute myocardial infarction.

Some patients survive for a long time following the onset of cardiogenic shock in acute myocardial infarction, and in these patients we have often observed the presence of right ventricular hypertrophy at autopsy. This clinical observation prompted a systematic retrospective study.

MATERIAL AND METHODS

This hospital has a coronary care unit (CCU) with 7 beds. Its general diagnostic criteria and therapeutic policy have been described elsewhere (1). All clinical information is collected into data records, which formed the basic material of this study. During Jan. 1968 – March 1970, 33 patients with proven acute myocardial infarction died in the CCU after having been in cardiogenic shock.

The criteria of shock in this study were systolic blood pressure below 90 mmHg in association with mental confusion, cold extremities and, when measured, oliguria. The patients were treated with 1) parasympatholytic drugs if bradycardia was present; 2) rapid infusion of 5.5% glucose if this was not contraindicated by raised central venous pressure or pulmonary oedema, 3) sodium bicarbonate infusion; and 4) norepinephrine or isoprenaline infusion. The survival time after onset of shock was calculated from the first recording of systolic blood pressure below 90 mmHg until the time of death.

In 30 of the 33 patients an autopsy was performed. Papillary muscle rupture was found to be contributory cause of death in patients, who were excluded for this reason. The studied group therefore consisted of 28 patients, in whom the pathologist had routinely made subjective estimation of 1) the size of the infarcted area of left and right ventricles and 2) the presence of any hypertrophy or dilatation of the cardiac chambers.

RESULTS

The distribution of the survival times after onset of shock showed a very skew pattern. It ranged from 0 to 4 hours in 17 patients and from 8 to 240 hours for the other 11.

The autopsy demonstrated an acute infarction of the left ventricle in all cases, with involvement of the right ventricle in three cases.

Of the 28 patients 13 were found to have right ventricular hypertrophy (RVH). The survival time after onset of shock for these 13 patients was 49 ± 21 (S.E.) hours, which is significantly longer than $6 \pm$ [illegible] hours for the other 15 patients without RVH ($p < 0.05$). If the delay between onset of infarct symptoms and shock was added to the survival time after onset of shock, the relationship between time and RVH disappeared ($p > 0.05$).

The heart weight was increased in patients with RVH, 611 ± 28 (S.E.) against 473 ± 24 g for the remainder and in those with left ventricular hypertrophy 515 ± 15 g against 395 ± 40 g for the remainder which are significant differences. In contrast there was no correlation between RVH and left ventricular hypertrophy, previous myocardial infarcts, or right ventricular dilatation.

Macroscopic lung pathology was found in only 3 patients. Even after exclusion of these 3 pa-

Table I *Cardiogenic shock studied in 28 patients*

RVH = Right ventricular hypertrophy RVD = Right ventricular dilatation. LVH = Left ventricular hypertrophy LVD = Left ventricular dilatation. P = Including previous infarct. a = Anterior i = Inferior r = Right ventricular involvement

Pat. no.	Sex	Age (y)	Admission delay (h)	Time between admission and onset of shock (h)	Survival time after onset of shock (h)	Died in shock	Smoker or previous smoker	At autopsy: Heart weight (g)	RVH	RVD	LVH	LVD	Infarct. size	Site of infarct.	Pulmonary pathology
060	♂	62	1	0	3	Yes	—	545	—	—	Yes	—	70[P]	i	No
136	○	80	3	0	1	Yes	S	450	—	—	Yes	—	70[P]	i	No
145	♀	69	14	45	–	Yes	—	450	—	—	Yes	—	75[P]	a	No
151	♂	67	2	1	0	Yes	—	610	Yes	—	Yes	—	90[P]	a	No
217	♂	61	<5	57	40	No	S	460	Yes	Yes	Yes	Yes	80[P]	i, r	No
266	♂	67	1	34	11	Yes	—	410	—	—	Yes	—	35	i, r	No
021	♂	70	5	12	8	Yes	S	630	—	Yes	Yes	Yes	80[P]	i, r	No
114	♂	75	8	7	13	Yes	S	650	Yes	Yes	Yes	Yes	?[P]		*
171	○	76	1	9	17	Yes	S	650	Yes	—	Yes	Yes	65[P]	a, i	No
189	♂	79	10	3	1	Yes	S	520	—	—	Yes	—	65	a, i	No
239	♀	61	3	12	4	Yes	S	360	—	—	—	—	65		No
282	♂	90	1	0	111	Yes	S	590	Yes	—	Yes	—	70	a, i	*
310	♂	80	8	11	27	Yes	S	605	—	Yes	Yes	—	55[P]	a	No
313	♀	77	14	0		Yes	—	340	—	Yes	—	Yes	60[P]	a	No
349	♂	59	1	0	4	Yes	—	460	—	—	Yes	—	70		No
353	♂	73	5	13	16	Yes	S	730	Yes	—	Yes	—	100[P]	a	No
379	♂	53	1	1	2	Yes	S	470	Yes	—	Yes	—	80[P]	a	No
463	♀	74	5	12	3	Yes	—	790	Yes	—	Yes	—	70[P]	i	No
507	♂	73	7	4	144	No	S	630	Yes	—	Yes	—	90[P]	i	No
534	♂	77		4	2	Yes	S	500	—	—	Yes	—	85[P]	a, i	No
538	○	74	7	0	84	Yes	S	600	Yes	—	Yes	—	30	i	No
577	♀	60	3	40	3	Yes	S	390	—	—	—	—	90	a, i	No
697	♀	73	7	7	18	Yes	—	330	—	—	Yes	—	80[P]	a, i	No
726	♀	71	6	10	1	Yes	—	350	—	—	—	—	80[P]		No
045	♂	94	4	1	1	Yes	—	570	Yes	—	Yes	—	100[P]	a	No
067	♂	60	4	0	2	Yes	?	530	—	—	—	Yes	?[P]	i	No
142	♂	75	7	8	1	Yes	S	690	Yes	—	Yes	—	70[P]	a	N
193	♂	63	5	0	1	Yes	?	400	Yes	—	—	Yes	?[P]	i	*

Per cent of left ventricular myocardium.
* Emphysema.
Minor pulmonary emboli and infarction.

tients the survival time after onset of shock was *significantly longer for patients with RVH than* for those without. Sixteen patients were or had been smokers, but they were not overrepresented in the RVH group

During the same period 14 patients with acute myocardial infarction were autopsied, who had been in pulmonary oedema but not in shock. Seven of these patients were found to have RVH at autopsy but their time of survival did not differ significantly from those without RHV

DISCUSSION

The value of these results is limited by the fact that RVH was not objectivized by measuring the wall thickness or excising and weighing the right ventricle. On the other hand when the pathologist has no knowledge of the present hypothesis, as in this study the findings may yet have some value.

The RVH found to be associated with a longer survival time in shock may either have existed before the infarction or have developed following it. The common causes of RVH, left heart failure and pulmonary disease, were not found to be overrepresented in the RVH group when measured as previous myocardial infarcts or left ventricular hypertrophy and a history of smoking or gross lung pathology respectively. Therefore no cause of a pre-existing RHV has been found. The other possibility is that the RHV developed following the infarction or even the onset of shock. The development of RVH seems to be rapid in experimental pulmonary artery constriction, as shown in cats by Spann et al. (2), *where the weight of the right ventricle was nearly* doubled in two days. The pressure load on the right ventricle in acute myocardial infarction would reasonably seem to be much less than in these experiments, but the load necessary for producing RVH is not known. The equal incidence of RVH in the shock and the pulmonary oedema groups does not support the possibility of RVH developing after the onset of shock.

To sum up, an association between RVH and longer survival time in shock but not in pulmonary oedema has been found following acute myocardial infarction. This has, however to be confirmed before any conclusions can be drawn, and then comes the problem whether the RVH has a causal relationship to the increased survival time in shock.

ACKNOWLEDGEMENT

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IRON STORAGE IN ALCOHOLIC FATTY LIVER

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Abstract. Liver storage iron and its relationship to the degree of steatosis have been studied in 52 male alcoholics without cirrhosis of the liver. A control series of 20 hematologically normal males with uncomplicated gall bladder disease was also studied. The amount of storage iron was determined histochemically and chemically. Chemical iron was related to dry weight and to alkali-soluble protein. The degree of steatosis was determined by planimetric estimation of the amount of visible fat. The mean chemically determined liver iron concentration calculated on dry weight was significantly lower in alcoholics as compared with controls. With protein as base of reference the mean iron concentration of alcoholics was lower than that of controls but not significantly so. The liver iron concentration was unrelated to the degree of steatosis. Histochemically visible iron was found in parenchymal liver cells approximately in the same frequency in alcoholics as in controls. The amount of histochemical iron in liver parenchymal cells was unrelated to the degree of steatosis. Histochemical iron in Kupffer cells was, however, significantly more frequent in alcoholics as compared to controls. There was more histochemically visible iron in Kupffer cells of livers with marked steatosis. It is concluded that non-cirrhotic alcoholics consuming mainly distilled spirits do not show any increase of the total liver iron content irrespective of the degree of fatty liver. The increased occurrence of histiocytic hemosiderin in the alcoholics is suggested to be due to transfer of iron from injured parenchymal cells.

It is often believed that increased iron storage in the liver frequently occurs in alcoholics. However, in a recent study of chronic alcoholics consuming predominantly distilled spirits, liver iron was not increased (8). It is also a common conception that increased iron deposition often is present in liver disease (2, 19–22). Many authors have maintained that increased liver iron storage is common in hepatic steatosis (fatty liver) (7, 10) and especially so in alcohol-induced steatosis (4, 5, 17). Thus Dittrich (4) reported an incidence of evident siderosis of 69% in alcohol-induced steatosis, but only of 1% in unselected biopsy specimens.

In the above studies, however, due regard was not paid to other factors influencing the size of iron stores such as sex, blood loss, blood transfusion, iron administration or gastric resection. Furthermore, no acceptable control series were presented. Hence, no conclusions can be drawn from these studies regarding the occurrence of liver iron overload in alcoholic steatosis.

The aim of the present investigation was to study the relationship between liver iron storage and alcohol-induced steatosis. Only male alcoholics without a history of blood loss or iron medication were studied. The results were compared with those obtained in a control material of non-alcoholic males who had no or minimal steatosis. Iron was determined in liver biopsy specimens histochemically and by chemical analysis.

MATERIAL AND METHODS

No subject included in the study had a history of blood loss, had undergone gastric resection or had received blood transfusions or iron medication. Only males were studied.

The control series comprises 20 males with a mean age of 44 years (range 19–81 years). They were admitted for cholecystectomy because of uncomplicated gall bladder stone, but were otherwise healthy. There was no abuse of alcohol. The control series has been reported in detail elsewhere (8).

Fifty-two male alcoholics with a mean age of 51 years (range 24–70 years) were studied. None had cirrhosis of the liver. Their alcohol intake, calculated as 40% ethyl alcohol was at least 1 l per week for at least five years. The mean consumption of distilled spirits for the group was estimated at seven l per month. Wine consumption was moderate. At the time of investigation 23 of the patients were in a mental ward (Clinic IV, Lill-

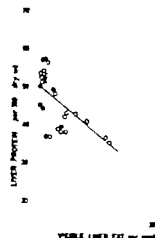


Fig 1 Relationship of liver protein concentration to the amount of visible liver fat in alcoholics.

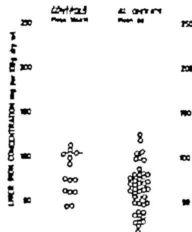


Fig 3 Liver iron concentration calculated on dry weight in controls and alcoholics. The broken horizontal lines represent mean values.

hagen Hospital) for chronic and acute alcoholism and 29 in somatic ward (Department of Medicine I, Sahlgren's Hospital). The cause of admission for the latter was in most cases medical complications of chronic alcoholism.

In the controls the biopsy specimen was taken from the margin of the right liver lobe at the operation (cholecystectomy). In the alcoholics percutaneous transthoracic biopsy was performed. The biopsy was made within 10 days after admission in most alcoholics. In both groups the biopsies were performed by needle aspiration technique. Needles with an internal diameter of 1.6 mm were used.

The methods used for the determination of liver nonheme iron, alkali-soluble liver protein, visible liver fat, as well as for the quantification of stainable iron in liver parenchymal cells and in Kupffer cells, are the same as described previously (8).

The term visible fat in the present study denotes vacuoles in liver cell cytoplasm. When fat vacuoles constituted less than 0.5% of the area of the section the steatosis is regarded as minimal, 0.5 to 2.5% of visible fat as slight steatosis, 2.6 to 6.0% as moderate steatosis and more than 6.0% of visible fat as marked steatosis.

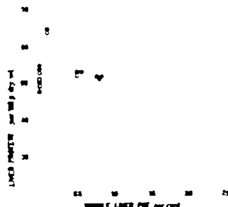


Fig. 2 Liver protein concentration in livers with no or slight steatosis. Filled circles represent alcoholics, open circles represent controls.

RESULTS

Visible liver fat and liver protein

Of the 20 control subjects 10 had no, 4 minimal and 6 slight steatosis. In alcoholics 5 had no steatosis, 3 minimal, 13 slight, 9 moderate and ... marked steatosis.

The mean alkali-soluble protein content of the liver in 17 controls was 56.1 ± 1.2 g/100 g dry weight. In 47 alcoholics the mean protein concentration was 44.8 ± 1.3 g/100 g and was significantly lower than that of controls ($t = 4.7$ $p < 0.001$). The mean protein concentration in alcoholics with no or slight steatosis (51.1 ± 1.4 g/100 g) was also significantly lower than that of controls ($t = 2.7$ $p < 0.01$).

In alcoholics with all grades of steatosis there was a negative regression of protein on visible fat (Fig. 1). The equation of linear regression was, protein (g/100 g) = 50.1 − 0.82 visible liver fat (%). The correlation coefficient was 0.622 ($p < 0.001$) and the residual standard deviation 7.3 g protein/100 g at a mean protein concentration of 44.8 g/100 g.

In the controls there was no significant relationship between protein concentration and liver fat (Fig. 2): the mean protein concentration of

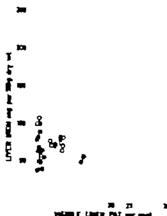


Fig. 4. Liver iron concentration calculated on dry weight related to the amount of visible liver fat in alcoholics.

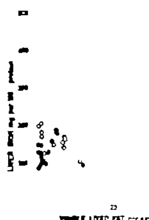


Fig. 6. Liver iron concentration calculated on protei related to the amount of visible li er fat in lcoholics.

those with visible fat (56.4 ± 1.5 g/100 g) did not differ from the mean of those without visible fat (55.7 ± 2.1). Nor was there any significant relationship between li er fat and liver protein in alcoholics with no to slight steatosis (Fig. 2).

Liver iron concentration

Calculated on dry weight (Fig. 3) the mean iron concentration of the 20 controls was 104 ± 11 mg/100 g. The mean of those with visible fat (103 ± 13) did not differ from the mean of those without visible fat (106 ± 19).

The mean liver iron concentration in 47 al coholics (68 ± 5 mg/100 g dry weight) was significantly lower than that of controls ($t = 3.5$, $p < 0.001$). There was no significant regression of liver iron on visible fat (= −0.2, $p > 0.20$) (Fig. 4). The mean liver iron concentration of alcoholics with marked steatosis (62 ± 6) was slightly lower than the mean of the others (73 ± 7), but not significantly so.

Calculated on protein (Fig. 5), the mean iron concentration of the 20 controls was 186 ± 21 mg/100 g. The mean liver iron concentration of the 47 alcoholics was 148 ± 11. This mean was not significantly lower than that of controls ($t = 1.7$, $0.10 > p > 0.05$). There was no regression of the liver iron concentration/100 g protein on liver fat (= 0.03) (Fig. 6).

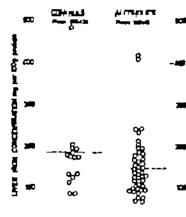


Fig. 5. Liver iron concentration calculated on protein in controls and alcoholics. The broken horizontal lines represent mean values.

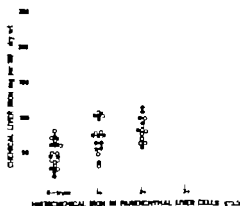


Fig. 7. Relationship between chemical liver iron calculated on dry weight and histochemical iron in parenchymal liver cells. Open circles represent alcoholics and closed circles represent controls.

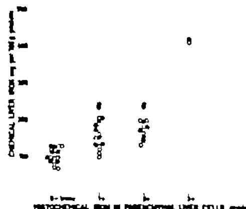


Fig 8 Relationship between chemical liver iron calculated on protein and histochemical iron in parenchymal liver cells. Open circles represent alcoholics and closed circles represent controls.

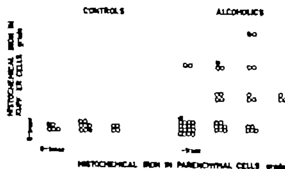


Fig 10 Relationship between histochemical iron in Kupffer cells and in parenchymal liver cells.

Histochemical liver iron

In parenchymal liver cells stainable iron of grade 1+ or more was present in 15 of 20 controls (75%). Eight had stainable iron of grade 1+ five 2+ and two 3+. In alcoholics stainable iron in parenchymal liver cells of grade 1+ or more was present in 32 of 52 subjects (62%). Fifteen had grade 1+ twelve 2+ and five 3+. The relationship between the grade of histochemical iron in parenchymal liver cells and chemical liver iron values is shown in Figs. 7 and 8. There was a considerable overlap between the non-heme iron values of the different histochemical gradings.

As seen in Fig. 9 the distribution of histochemical gradings of parenchymal liver cell iron in alcoholics who had marked steatosis was not significantly different from those with no or slight steatosis or from controls.

In Kupffer cells stainable iron was present in 3 of 20 controls (Fig. 10). These also had stainable iron in parenchymal liver cells. Significantly more (22 of 52) alcoholics had stainable iron in Kupffer cells as compared to controls ($\chi^2 = 4.75$ $p < 0.05$). In two alcoholics stainable iron was present in Kupffer cells although they had no iron in parenchymal cells. Disproportionate amounts of stainable iron in Kupffer cells were present in many other alcoholics (Fig. 10). Stainable iron of grade 1+ or more in parenchymal and/or Kupffer cells was present in 35 of 52 alcoholics (67%).

The relationship between liver fat and the grade of histochemical iron in Kupffer cells is shown in Fig. 11. Alcoholics with moderate or marked steatosis had significantly more iron in Kupffer cells than those with no or slight steatosis ($\chi^2 = 5.34$ $p < 0.05$).

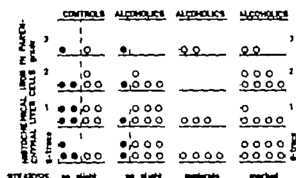


Fig 9 Relationship between histochemical iron in parenchymal cells and the degree of steatosis. Filled circles represent liver biopsy specimens without fat vacuoles.

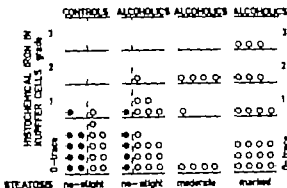


Fig 11 Relationship between histochemical iron in Kupffer cells and the degree of steatosis. Filled circles represent liver biopsy specimens without fat vacuoles.

COMMENT

Stainable liver iron was found in 75 % of the present control males. This is in accordance with the results in an earlier study of male controls (21) and with the results of MacDonald and Pechet (9) who found stainable hepatic iron in 53–80 % in unselected autopsy series from different areas of the world. The lower frequency of stainable liver iron in some other reports (4 19) might be due to many factors. The most important cause of the divergent results is probably differences in the composition of the materials. Earlier blood loss and gastric resection may grossly diminish the size of the iron stores. Women, especially menstruating women (21), have a lower frequency of histochemically demonstrable liver iron. Therefore due regard must be paid to these factors if a group of alcoholics (who most often are males) is to be compared with an unselected group of control subjects. In the present investigation only males were studied and none had a history of previous blood loss or iron therapy With respect to the size of iron stores, accordingly the alcoholics were comparable to our control subjects. The results have shown that stainable iron was not more frequent in alcoholics (67 %) than in controls (75 %). There was no significant relationship between the degree of steatosis and the grade of histochemical iron in parenchymal liver cells. However in Kupffer cells, stainable iron was more common in alcoholics than in controls. Furthermore, higher grades of iron in Kupffer cells were more common in livers with moderate or marked steatosis than in livers with no or slight steatosis. The frequent finding and the higher gradings of histochemical iron in Kupffer cells in alcoholics with marked steatosis may be due to parenchymal liver cell damage with release of ferritin and phagocytosis by the Kupffer cells. Ferritin in serum has been demonstrated in experimental liver injury (15) and in human hepatocellular disease (16).

The mean chemical liver non-heme iron concentration of the present control series is in accordance with that obtained in a previous control series (20 21) comprising males with uncomplicated gallstone disease or peptic ulcer Mean values f the same order have also been obtained in males killed by accident (11 12, 13 18). The mean iron concentration calculated on dry weight was significantly lower in the alcoholics as compared with controls. In the former the iron concentration did not rise with increasing degree of steatosis. The study indicated rather that there might be a negative regression between the liver iron concentration and the amount of liver fat. However most patients with marked steatosis had an enlarged liver at palpation, which might have caused a dilution of the iron concentration if the calculation is made with weight as reference base whereas the total amount of liver iron could remain unchanged. Calculated on dry weight the decrease of iron concentration should be considerable because of the low water content of fat. Therefore alkali-soluble protein was also used as a reference base for the calculation If we assume that the total content of protein in the liver is unchanged with different degrees of steatosis, protein as reference would give more reliable information on changes in the total liver iron content. With protein as reference the mean iron concentration of alcoholics was lower than that of controls, but not significantly so. If, however total liver protein is decreased in steatosis, iron related to protein as reference would give too high values. The results indicated (Fig. 4) that the protein content of alcoholics with no or slight steatosis was significantly lower than that of controls, suggesting that the protein content of alcoholic livers may be decreased. Thus in no way did the present study show an increased iron concentration in alcoholics. The results rather indicate decreased liver iron in alcoholics.

It has been suggested that histochemically visible iron might be increased in liver disease because of increased transformation of storage iron to hemosiderin as consequence of decreased apoferritin synthesis (6) Fig. 7 which shows the relationship between the grade of histochemical iron in parenchymal liver cells and chemical liver iron concentration calculated on dry weight, might support this suggestion, the mean iron concentrations of alcoholics with grade 1+ and 2+ were significantly lower than those of controls in the same gradings. This may however be due to low iron concentration values in alcoholics as a consequence of dilution with fat. With protein as reference (Fig. 8) the values of alcoholics were more in accordance with those of controls in the same histochemical gradings. the

data did not show that the relationship between histochemical iron in parenchymal liver cells and chemical iron is significantly altered in alcoholic liver disease.

Increased liver storage iron is common in Bantus in South Africa. The main cause is considered to be ingestion of alcoholic beverages containing large quantities of iron derived from iron containers used for brewing (3). Much iron may be present in wine (1 14) and abuse of such wines might be a cause of increased iron storage. In this series there was no significant relationship between the liver iron concentration and the estimated amount of wine consumed. However the present alcoholics ingested mainly hard liquor and the wine consumption was comparatively low

ACKNOWLEDGEMENT

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HEMOLYSIS IN MITRAL VALVULAR DISEASE AND MITRAL BALL VALVE PROSTHESES

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Abstract The degree of hemolysis has been evaluated in a series of 51 non-selected patients with mitral valvular disease and 21 cases with prosthetic mitral valves; the findings were compared to the findings in 97 patients with aortic valvular disease and 56 with aortic ball valves. The destruction of red blood cells was predicted from the serum levels of lactic dehydrogenase. The frequency and the degree of hemolysis were found to be quite similar in patients with lesions of the aortic and mitral valves. As previously shown in patients with aortic ball valves, the degree of hemolysis was highly dependent on the type of prosthesis inserted, whereas paravalvular leakage seemed to be less important. Our findings suggest that the direct traumatic effect of the prosthesis on the erythrocytes has a greater significance for hemolysis than the turbulence of the blood stream.

The general opinion is that mitral valve prostheses provoke less hemolysis than prosthetic aortic valves (7). However only a few reports of a rather limited number of patients with lesions of the mitral valves have been published (1–7). Therefore we evaluated the red blood cell destruction in a non-selected series of patients with mitral valvular disease and valve prostheses. The results were compared to those found in a simultaneously studied series of patients with aortic valvular disease and valve prostheses.

Red blood cell destruction was predicted from the level of serum lactic dehydrogenase activity (LDH) as described previously (4). The LDH levels were quite similar in patients with mitral and aortic valvular diseases, and in cases with mitral and aortic valve prostheses. As in patients with prosthetic aortic valves (3), the type of mitral valve inserted seemed to be a major determinant of red blood cell destruction.

MATERIAL AND METHODS

Fifty-one consecutive, non-selected patients with mitral valvular disease and 21 with prosthetic mitral valves admitted during 1969 were studied. For comparison the degree of hemolysis was evaluated in 97 patients with aortic valvular disease and 56 with aortic ball valves seen during the same period. Patients with lesions of both mitral and aortic valves were excluded from the study. The pressure gradients across the diseased valves and the degree of regurgitation were determined by heart catheterization and angiocardiography.

Patients with acute myocardial or pulmonary infarction, severely impaired liver function, or elevated levels of serum transaminases and phosphatases were not included in the material. No case of megaloblastic anemia was seen in this study; the serum levels of vitamin B_{12} and folic acid were measured in patients with more severe anemia. The time elapsed since insertion of the prosthetic valves varied from two months to several years, on an average eight months.

The total serum LDH activity was determined according to Wroblewski and LaDue (6), the upper normal limit in our laboratory being 200 U/l (µmol min/l). The erythrocyte destruction rate was predicted from the LDH levels as described elsewhere (4). LDH less than 200 U/l indicated normal red blood cell survival, whereas values exceeding 500 U/l suggested a destruction of twice the normal or more.

RESULTS

Fig. 1 shows the main hematological data in the different groups of patients with mitral valvular disease and valve prosthesis. LDH levels above normal were found in 35% of the unoperated cases and 95% of patients with valve prostheses. Hemolysis presumably exceeded twice the normal only in two unoperated patients, both suffering from mitral regurgitation. In 21

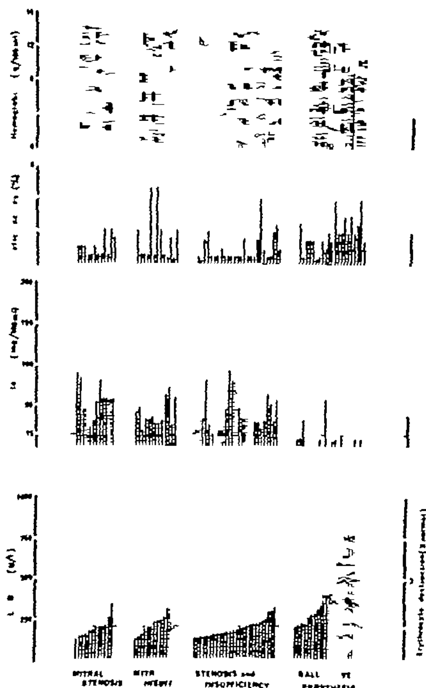


Fig. 1. The main hematological findings. The individual values are arranged according to increasing LDH levels in each group. Patients with iron deficiency (○) and ball valve incompetence ( ) are indicated. The broken lines give the normal tunes of LDH and haptoglobin.

prosthetic mitral valves LDH increment exceeding 500 U/l was seen in nine, or nearly 45 %.

Paravalvular leakage was present in three cases, their LDH value did not differ from the levels in patients with competent valve prostheses.

In general the hemoglobin concentration decreased with increasing hemolysis as predicted from the LDH levels. Hb levels below 1 g/100 ml were found in three unoperated and five operated cases, the frequency of anemia thus being four times greater in the operated than in the unoperated patients. Severe hemolytic anemia developed in one patient with a competent mitral valve prosthesis, the LDH increased to 1 390 U/l and the halflife of her own ^{51}Cr-labelled erythrocytes was shortened to nine days. A remission occurred during bed rest and iron treatment.

A comparison of the LDH levels in aortic and

mitral valvular disease is shown in Table I There was no difference in LDH levels between unoperated patients with aortic and mitral valvular disease. Further the increment of LDH following valve replacements was similar in the two groups.

Fig. _ shows that hemolysis, as predicted from the serum LDH levels, depended on the type of prosthetic valve inserted. In the patients with Beall valve prostheses the mean LDH was 6_5 U/l compared to _75 U/l in cases with Starr Edwards valves. The mean observation time since operation was six months in the patients with Beall valves and nine months in the group with Starr Edwards valves. However the degree of hemolysis remained remarkably constant in the individual patients as time passed, and therefore the different observation time scarcely influenced the results. Moreover all five patients developing anemia had Beall valve prostheses implanted.

Several cases both in the unoperated and the operated group were found to have iron deficiency However the significance of the iron deficiency could not be properly evaluated because more than half the patients received iron supplement during the observation period.

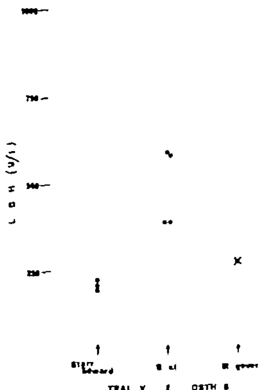


Fig 2. Hemolysis in patients with different types of mitral ball valve prostheses.

DISCUSSION

The present study showed that both frequency and degree of hemolysis were quite similar in patients with mitral and aortic valvular disease and following mitral and aortic valve replacement. Even cases with severe anemia occurred with the same frequency in the two groups of patients. This is somewhat in disagreement with findings reported by others (1 7).

Table I *LDH levels in patients with aortic and mitral valvular disease and prosthetic valves*

	No. of pats.	Mean LDH	Percentage of cases with LDH (U/l) Less than 200	200–50	Above 500
Valvular heart disease					
Aortic	97	193	63	37	0
Mitral	51	207	63	33	4
Valve prosthesis					
Aortic	56	450	4	62	34
Mitral	21	492	4	53	43

Obviously the red blood cell destruction depends on the type of mitral valve inserted, a finding in accordance with our previous study of aortic ball valve prostheses (3). Most authors consider paravalvular leakage to be a very important factor provoking hemolysis (1 5 7) this was not confirmed in our study

Our observations raise doubt about the generally accepted theory that turbulence of the blood stream is a main determinant of hemolysis in patients with valvular heart disease and valve prostheses. If so, hemolysis should be expected to be much heavier in aortic than mitral valve prosthesis, and especially severe in regurgitation due to leakage. However we found that hemolysis was less influenced by the site of the prosthesis and paravalvular leakage than by the type of prosthesis inserted.

We suggest that the direct traumatic effect of the prosthetic valve on the erythrocytes probably is most important in this respect.

ACKNOWLEDGEMENT

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THE RAPID LOWERING OF HAEMATOCRIT BY EXCHANGE TRANSFUSION OF RHEOMACRODEX® DEXTRAN 40

Its Use in Polycythaemia, Cor Pulmonale and Ischaemic Disease

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Abstract. Exchange transfusion using dextran 40 has been described in 31 patients. This would appear to be safe and logical procedure for treating both primary and secondary polycythaemia where venesection would normally be considered. As much as 3.5 l of blood may be removed rapidly at time without harming the patient. The method may also be of benefit in thrombotic disease as it allow much larger loading dose of dextran 40 than could normally be given safely It is suggested that the resultant fall in haematocrit may be important in improving tissue perfusion despite loss of oxygen carrying capacity A clinical trial of the procedure in the various conditions mentioned is planned in the Island f Guernsey in conjunction with studies at Guy' Hospital, London.

Venesection has an established place in the management of primary polycythaemia being the most satisfactory way of rapidly lowering blood viscosity thereby reducing peripheral resistance and improving tissue perfusion. Its use in the secondary polycythaemia of hypoxic pulmonary disease is controversial (23) but it can be argued that the increase in red cell mass serves no useful purpose, causes a fall in oxygen supply to the tissues and may well be of great importance (together with hypoxia, acidosis and the associated increase in circulating blood volume) in actually precipitating heart failure (11) and thromboembolic complications. Various workers have advocated venesection in cor pulmonale (2, 29 33). Venesection is not, however, without its dangers and may provoke hypovolaemic shock and venous thrombosis. Other methods of lowering the haematocrit have, therefore, been suggested such as by prolonged oxygen therapy (5) and by drugs (27). These methods, although ingenious, have the drawback of being time consuming and thereby not applicable to the emergency situation. Exchange transfusion with dextrose has once been used with advantage in a neonate with polycythaemia associated with the respiratory distress syndrome (10). Theoretical work by Hint (19) has suggested that the optimum haematocrit is in the region of 30 %. At this low figure, he argues, tissue perfusion is sufficiently rapid because of decreased viscosity that, despite a reduction in oxygen carrying capacity the actual oxygen available to the tissues is increased.

With these considerations in mind, therefore, some patients with relatively high haematocrits were bled. Simultaneously a transfusion of dextran 40 was given in order to maintain a more or less constant circulating blood volume and to allow the rapid and safe venesection of a large volume of blood. The patients were suffering from a variety of conditions in which poor generalised or regional perfusion (either at a peripheral or pulmonary level) was thought to be important such as cor pulmonale, polycythaemia, pulmonary emboli, strokes, coronary thrombosis and peripheral vascular disease.

The choice of dextran 40 was made firstly because it is an osmotically active plasma substitute and stays within the vascular compartment long enough for haemodynamic equilibrium to be restored (1 17 18) secondly because of its reputed ability to improve tissue perfusion by decreasing viscosity (16, 30). Many studies in animals indicate that dextran 40 improves perfusion in the presence of vascular occlusion (8, 12, 14 22) and somewhat less convincing clinical evidence that dextran 40 may be of use in man in such conditions as coronary occlusion (21 24 32), acute strokes (15), mesenteric occlusion (9), acute

Table I *Case 2. Changes before and five days following exchange transfusion*

	Hb (g)	PCV ( )	PaO (mmHg)	PaCO2 (mmHg)	B P (mmHg)	Urea (mg)	Peak flow (l min)	Weight (kg)	Chest X-ray
Before	16.7	53	6_	41	150/80	_6	110	68.04	Heart slightly enlarged. Pulmonary congestion
After	11.8	38	77	33	120/70	18	130	65.77	Heart normal size no pulmonary congestion

arterial insufficiency of the limbs (4 8) and acute thrombophlebitis (6, 7 31). Results might have been better if simultaneous venesection had been employed which would have allowed a much higher loading dose of dextran 40. Another reason for the use of dextran 40 was that claims have been made that it has anti-thrombotic properties (25) which would have clear advantages. It is of interest to note that when volunteers were given a 500 ml exchange transfusion of dextran 70 experimentally they were subsequently found to have greatly diminished platelet adhesiveness (3).

METHODS

Blood is taken from one arm after enous tourniquet is applied and intravenous heparin 10 000 U given into an occluded ein. A dextran 40 (10 Rheomacrodex®) in normal saline or dextrose) infusion is given into the other arm. The procedure is carried out rapidly and the rates of the venesection and infusion are usually kept similar. Between 500 ml and _5 l have been exchanged depending on the initial haematocrit. In cases of hypochromic polycythaemia some of the blood may be replaced by fully haemoglobinized blood. In occlus ascular disease the drip is often kept running slowly for up to two weeks giving dextran 40 usually with heparin (aiming to keep the clotting time at about three times normal). On the day of the procedure high fluid intake is encouraged in order to avoid possible renal complications (see below).

CASE REPORTS

Case 1

A 69-year-old man (cor pulmonale, secondary polycythaemia, deep vein thrombosis and pulmonary embolism).

This patient, a retired -naval commander, was "respiratory cripple who had been steadily deteriorating for more than two years. H as virtually housebound because of breathlessness.

In May 1968 he had an ttack of sudden left-sided pleuritic pain which lasted three hours. The following day his right leg became painful and ery swollen. T o days later he was admitted to hospital in extremis ith right-sided chest pain. On examination he was grossly cyanosed, breathless and disorientated. He had signs of severe emphysema and right entricular failure with triple rhythm, engorged neck eins and peripheral oedema. There was evidence of extensi deep vein thrombosis of the right leg. A diagnosis of pulmonary embolism was made and he was treated with 28% oxygen, intravenous heparin, hydrocortisone and frusemide. Despite these measures the patient's condition steadily deteriorated. One hour after admission one litre exchange transfusion with dextran 40 as performed as an emergency procedure. Within three hours there was dramatic improvement. The patient became pink at rest without xygen, he was no longer short of breath and he was then rational and alert. The signs of cardiac failure had improved ith loss of triple rhythm and a fall in jugular enous pressure. Having remained well for 4 hours he then had another attack of severe pleuritic pain. For f days the patient's condition as critical with intractable cardiac failure. A further one litre exchange transfusion was performed with the same rapid improvement as before. Over the next few days the patient was mobilized and was able to walk and climb stairs without distress. He stated that his exercise tolerance had greatly improved and that he felt better than he had for years. He was able to lie flat in bed w thout breathlessness. At this time his Hb had fallen from 19.2 g to 13.9 g and the haematocrit from 65 to 42. Eight days after the second exchange transfusion he developed an overwhelming staphylococcal bronchopneumonia and died three days later despite intensive treatment.

Case 3

A 70-year-old man (chronic bronchitis, emphysema, cor pulmonale).

This patient as suffering from severe progressi re spiratory disease. He was admitted in May 1970 at which time there was irreversible airways obstruction but no evidence of pulmonary infection. Cor pulmonale was well controlled on Natirelle digitalis one tablet daily and cyclopenthiazide 'K' 0.5 mg daily. No change was made in his treatment throughout the period of observation. He was severely orthopnoeic and required four pillows at night. He complained of severe shortness of

Table II. *Results of exchange transfusions*

No. of cases	No. of exchanges	Venesected (ml)	PCV % Before	PCV % After	Early clinical result	Follow-up
1. *Cor pulmonale*						
15	21	500–3 750	47–69	35–55	14 improved 1 no improvement	10 alive and well 1 to 24 mo later. 1 deteriorated over 6 mo. but improved by further exchange. 3 died of pneumonia 16 d, 2 mo and 4 mo. afterwards
2. *Myocardial infarction*						
6	7	500–1 000	44–51	33–40	4 improved. 1 died after 4 weeks peritoneal dialysis for bilateral renal emboli. 1 died within 2 h of intractable shock	3 alive and well 10 mo. later
3. *Chronic cerebro-vascular disease*						
2	2	1 000	43–51	35–41	Subjective improvement only	Both gradually improved over 2 y
4. *Acute cerebro-vascular disease*						
6	8	500–1 000	43–53	30–45	All improved	5 alive and well 14 mo later. 1 died of myocardial infarct after 10 mo.
5. *Pulmonary infarction*						
4	4	500–950	47–65	35–42	All improved	alive and well, 2 and 10 weeks afterwards. 1 died of pneumonia after 16 d. 1 died of myocardial infarct after 2 mo
6. *Acute peripheral vascular disease*						
4	4	500–1 000	43–51	38–47	All improved	Improvement maintained in 2 over 3 mo.-period. 2 died of myocardial infarctions 6 mo. and 12 mo. later

breath, even on climbing one flight of stairs slowly. On examination he appeared plethoric and cyanosed with warm periphery. The pulse was regular at 70/min and the B.P. 150/80 mmHg. The jugular venous pulse was not raised and no peripheral oedema was present. The liver was palpable three inches below the right costal margin. The apex beat was not palpable and the heart sounds were distant. Chest movements were poor with poor air entry in all areas. A few coarse expiratory rhonchi were present, together with some basal rales. An elective 2 000 ml exchange transfusion of dextran 40 was performed over 75 min. Following the procedure the patient's exercise tolerance had improved and he was no longer orthopnoeic. On the day of the procedure the patient passed over 6 l of urine with a fluid intake of 3.0 l. Re-examination showed an absence of basal rales. Changes were noted before and five days following the exchange transfusion (Table I).

RESULTS

In 37 exchange transfusions there have been no alarming side-effects such as hypo- or hypertension, cardiac failure or allergic reactions. Acute renal failure did occur in one patient who was having numerous systemic and pulmonary emboli following an antero-septal myocardial infarct. It was thought probable that bilateral renal emboli had occurred but the possibility of an "osmotic nephropathy" following dextran 40 was also considered (9, 13, 20, 26). The precise cause of the renal failure was not fully established because permission for an autopsy was not granted (Table I).

It was feared that replacing blood venesected

by the same volume of dextran 40 might produce circulatory overload in view of the temporary ability of dextran 40 to increase the circulation by as much as twice the infused volume of dextran 40. This theoretical hazard has not, however been encountered but could readily be prevented by giving, say half the quantity of dextran 40 at the time of venesection and the rest more slowly.

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PLATELET ADHESIVENESS IN PATIENTS WITH ISCHAEMIC HEART DISEASE

An Assessment By Three Whole-blood Methods

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Abstract. Platelet adhesiveness was estimated by three commonly used whole-blood methods in 50 patients with previous myocardial infarction and 50 matched controls. No evidence of increased platelet adhesiveness in these patients with ischaemic heart disease was obtained. No correlation was found between platelet adhesiveness and the intravenous glucose tolerance test, nor with serum cholesterol and triglycerides.

Platelets are presumably involved in the arteriosclerotic degenerative process. They are definitely involved in the major complication of this condition, i.e. thrombus formation. Less is known, on the other hand, about whether their role at this stage is an active or a passive one. In the former case one might expect altered platelet behaviour in these patients, and several methods have therefore been developed to assess platelet behaviour e.g. to measure platelet adhesiveness, aggregation as well as electrophoretic mobility.

Platelet adhesiveness, or stickiness, involves the estimation of platelet loss when blood or platelet rich plasma with or without the addition of adenosine diphosphate is brought into contact with a foreign surface—usually glass—for a certain period of time. The disappearance of platelets is usually expressed in per cent of the original number of platelets. Several methods have been involved and some comparisons between them have been made (9 10 13).

As conflicting results have been obtained as regards findings of increased platelet adhesiveness in patients with ischaemic vascular disease (14), we considered it of value to estimate platelet adhesiveness in patients with ischaemic heart disease (IHD) and healthy control subjects matched for sex and age by several methods in common use. Adhesiveness in these patients, estimated by Hellem's adenosinediphosphate-induced platelet adhesion method, has previously been found not to differ from the values obtained from control subjects in our laboratory (14).

As this method had not been found to be related to adhesion values obtained in whole-blood methods, a similar study has been performed with three whole-blood methods differing from each other in several important aspects. The methods used in this study are Hellem's whole-blood method (8), the Wright rotator test (16) and Salzman's method (1). Furthermore an investigation was made of the relationship between platelet adhesiveness and carbohydrate and lipid metabolism.

MATERIAL

In each comparison the results from 50 patients who had previously been treated for their first acute myocardial infarction at the Medical Department, Serafimerlasarettet, Stockholm, were compared with those from 50 control subjects matched for sex and age (within 4 years). At least one month had elapsed since the discharge of the patients from hospital.

The diagnosis of myocardial infarction was accepted when at least two of the following three criteria had been fulfilled: typical history, typical ECG changes and/or characteristic pattern in serum enzymes (GOT, GPT and LDH). Patients with overt diabetes mellitus were excluded. No patient received anticoagulant therapy at the time of the investigation. The patients had routinely been advised to reduce their fat intake following discharge from hospital. The selection of the healthy control subjects was based solely on negative history of myocardial infarction, oppressive chest pain on effort, intermittent claudication and diabetes mellitus. Controls who were found to be hypertensive or overtly diabetic at the examination were excluded, as were those who showed signs of ischaemic heart disease on standard ECG

Table I. *Salient features of the three methods used in this study*

	Method		
	Wright	Salzman	Hellem
Blood specimen	Citrated	Native	Citrated
Temperature	Room	Body	Room
Delay[a]	5	0	15
Driving force	3 rpm	Vacuum	Motor-driven syringe
Contact surface	Glass flask	Glass beads 0.4 mm diam.	Glass beads 0.5 mm diam.
Delivery time	—	35	23.5″
Contact time	20′	Mean 3.5″	28.2″

[a] Interval between venepuncture and process for adhesion.

(leads I, II, III, VR, VL, VF CR_{1-6}) taken at rest. All subjects had platelet counts within normal limits.

The groups investigated for each of the three tests were drawn from a common pool consisting of 97 patients and 94 controls. As the tests for estimating platelet adhesiveness were introduced successively there is a considerable overlap several subjects being involved in all three comparisons. As soon as the results from 50 matched pairs fulfilling the criteria had been obtained, the investigation with that method was stopped.

METHODS

After an overnight fast without smoking, and following a rest of at least 20 min after arrival at hospital, venous blood samples for estimations of platelet adhesion, serum cholesterol and triglycerides were taken through a siliconised needle. Cholesterol and triglycerides were determined as described previously (3, 5). No drugs had been taken during the previous 24 hours.

The experimental details of the three methods used were as follows.

Modified Wright rotator test.

Within 5 min of venepuncture ml citrated blood are transferred into a conical pyrex flask fixed horizontally into a drum rotating at 3 rpm. After 20 min a sample for platelet count is taken from the flask.

Hellem's whole-blood method

Fifteen minutes after venepuncture citrated whole-blood is passed through a glass-bead filter not more than one week old, at a constant rate by a motor-driven syringe. The filter is made of polyvinyl tubing with an internal diameter of 5 mm and contains 5 g Ballotini 8 glass-beads (Glas Export AG, Liberec, Czechoslovakia). The mean collection time for 1 ml was 23.5 sec, giving a mean platelet contact time of 28.2 sec.

Salzman's method

Venous blood is drawn directly through a glass-bead filter not more than one week old, made of polyvinyl tubing, internal diameter 4 mm, containing 2.5 g Ballotini 9 beads (Glas Export AG, Liberec, Czechoslovakia) by a 7 ml vacutainer (Becton, Dickinson & Co., Rutherford, NJ, USA) containing Na_2EDTA. A collection time of 35 sec was used, giving a mean contact time of 3.5 sec.

Platelet adhesion as determined from duplicate estimations was expressed according to the formula.

$$\frac{\text{initial count} - \text{final count}}{\text{initial count}} \cdot 100\,\%$$

All tests were performed twice and the mean was taken as the final value.

Platelet counts when testing with Wright's method were performed by the method of Björkman (2). In the other methods these were obtained using an electronic particle counter (Celloskop 101, AB Ljungberg, Sweden). Adhesion values using the counter were repeatedly checked against values obtained from microscopic platelet counts. Some of the features of the methods used are presented in Table I.

Table II. *Results of platelet adhesiveness (Wright's method), IVGT cholesterol, and triglyceride estimations in patients with ischaemic heart disease (n = 50) and controls (n = 50)*

	Adhesion (%)	IVGT (k-value)	Cholesterol (mg/100 ml)	Triglycerides (mmol/l)
Patients				
Mean	29.8	1.30	283	2.23
S.D. ±	10.3		55	0.9
Range	17–83	0.46–2.77	184–419	0.87–4.38
Controls				
Mean	29.5	1.48	249	1.53
S.D. ±	9.4		49	0.8
Range	8–52	0.60–3.58	165–420	0.80–6.42
Signif.	N.S.	N.S.	$p < 0.01$	$p < 0.001$

Table III. *Results of platelet adhesiveness (Hellem's method), IVGT cholesterol and triglyceride estimations in patients with ischaemic heart disease (n = 50) and controls (n = 50)*

	Adhesion (%)	IVGT (k-value)	Cholesterol (mg/100 ml)	Triglycerides (mmol/l)
Patients				
Mean	29.6	1.20	285	2.30
S.D. ±	10.9		56	0.91
Range	5–65	0.46–2.77	184–419	0.87–4.38
Controls				
Mean	27.1	1.52	249	1.57
S.D. ±	9.9		49	0.91
Range	5–50	0.63–3.58	157–420	0.59–6.42
Signif.	N.S.	$p < 0.01$	$p < 0.001$	$p < 0.001$

When blood for platelet adhesion had been taken, 25 g glucose in 50% aqueous solution as injected within 3 min for the intravenous glucose tolerance test (IVGTT). Duplicate blood glucose estimations from capillary blood samples before and 20, 30 40 50 and 60 min after the injection were determined. The half-life ($t_{\frac{1}{2}}$) of blood glucose as determined by graphic extrapolation of these alues, and the result of the IVGTT was expressed as k-value derived from the formula 0.693 100 $t_{\frac{1}{2}}$ representing the disappearance of blood glucose in per cent per minute.

Correlations ere tested by conventional methods or by rank correlation according to Spearman hen dealing ith samples of an uneven distribution, g. k values. Differences between groups were calculated by ranking according to Wilcoxon in the case of samples ith uneven distribution, otherwise with Student' t-test. Significance as tested at the 5 1 and 0 1 % levels.

RESULTS

Wright's modified rotator test

There were 50 subjects both in the patient and in the control group, 42 men and 8 women in each group. The mean age of the patients was 55±6.9 (S.D.) and of the controls 53 years ±7 8.

The results including adhesiveness, the IVGT cholesterol and triglyceride estimations are shown in Table II. Furthermore, a presentation of the adhesion values is seen in Fig. 1

In Table II it is shown that the patients differ from the controls significantly in cholesterol and triglyceride levels but not as regards platelet adhesiveness and IVGT No significant correlations were obtained between platelet adhesiveness and the IVGT cholesterol or triglyceride levels.

Table IV *Results of platelet adhesiveness (Salzman's method), IVGT cholesterol and triglyceride estimations in patients with ischaemic heart disease (n = 50) and controls (n = 50)*

	Adhesion (%)	IVGT (k-value)	Cholesterol (mg/100 ml)	Triglycerides (mmol/l)
Patients				
Mean	47.3	1.27	283	2.12
S.D ±	17.3		55	0.89
Range	10–78	0 46–2.77	184–419	0.87–4.38
Controls				
Mean	43.6	1.52	242	1.48
S.D ±	16 8		45	0.83
Range	13–85	0.66–3.58	157–382	0.59–6 42
Signif.	N.S.	$p<0.05$	$p<0.001$	$p<0\,001$

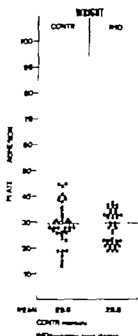


Fig 1 Distribution of platelet adhesion alues estimated by Wright method in 50 patients ith ischaemic heart disease (●) and 50 controls ( ).

Hellem's whole-blood method

The results obtained from the 50 patients and their 50 matched controls are given in Table III. The results of the platelet adhesiveness study are also demonstrated in Fig. .. There were 44 men and 6 women in both groups, with a mean age

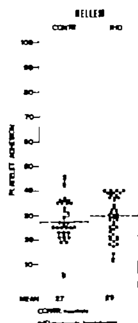


Fig. 2 Distribution of platelet adhesion values estimated by Hellem's method in 50 patients with ischaemic heart disease (●) and 50 controls (○).



Fig 3 Distribution of platelet adhesion values estimated by Salzman method in 50 patients with ischaemic heart disease (●) and 50 controls (○).

of 55 ± 10 for the patients and 54 ± 9 7 years for the controls.

From Table III it is seen that the patients with IHD differ significantly from the healthy controls in IVGT cholesterol and triglyceride levels but not as regards platelet adhesiveness. No significant relationship between platelet adhesiveness and the IVGT cholesterol and triglyceride values was found.

Salzman's test

In this test the two groups, patients and controls, consisted of 44 men and 6 women. The combined mean age of the patients was 56 ± 7.2 and that of the controls 54 ± 8 3 years.

The results are given in Table IV and those for platelet adhesiveness with this method are also shown in Fig. 3

Similarly to the results with the previous tests for platelet adhesiveness, no significant correlations were obtained between platelet behaviour as estimated by this method and carbohydrate metabolism (k values from the IVGT) and lipid metabolism as estimated by the cholesterol and triglyceride levels. The patients differed from the controls in having significantly lower k values and raised lipid values, but no difference was obtained as regards platelet adhesiveness.

DISCUSSION

No evidence of raised platelet adhesiveness in the patients with previous myocardial infarction studied by the three methods has been found. Nor was there any relationship between raised adhesiveness with these methods when compared with glucose tolerance or increased serum cholesterol and triglyceride levels.

The finding of raised serum cholesterol and triglyceride levels in the patients compared with the healthy controls is in agreement with previous investigations (4 6) as is that of lower glucose tolerance (15) The results given in Table II may seem misleading. In this comparison no significant difference between patients and controls was obtained, although the range given for the IVGT is identical with that for the other two studies, due to the fact that the two patients with he highest and lowest k values respectively happened to be the same in all three investigations. Yet the combined mean k value for the group is unusually high when compared with previous findings for patients with ischaemic heart disease at this hospital (15) This may either be due to chance or the observation of temporary improvement of intravenous glucose tolerance during the first year after an acute infarction (11) may provide an explanation.

When comparing patients and controls the absence of evidence of altered platelet behaviour estimated by the methods used should be compared with previous investigations which partly include the same methods. A review of some of the previous investigations in this field has been presented in connection with an earlier investigation (14). From the results obtained in this laboratory it would seem that there is no place for including estimations of platelet adhesiveness by the methods used in the routine investigation of patients with ischaemic heart disease. The present findings, on the other hand, do not contradict the possibility of altered platelet adhesiveness in the acute phase of a myocardial infarction, as reported by some authors, (1) nor of altered platelet behaviour as estimated by other methods (7)

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Congress Announcement

The Fourth International Congress of Phlebology will be held in Lucerne, Switzerland, 20 to 24 Sept. 1971

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Secretary PD Dr U Brunner Surgical University Clinic B, Zurich, Switzerland.

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515. Mortality trends in Sweden 1951–1968 with special reference to cardiovascular causes of death. By J. Anders Vedin, Claes-E. Wilhelmsson, Anne Marie Bolander and Lars Werkö.

516. Studies on peptic ulcer disease. With special reference to the effect of *l*-Hyoscyamine. By Anders Walan.

517. Intrinsic factor in tapeworm anaemia. By Juhani Salokannel.

518. Deficiency and absorption of iron in man. By Sverker Höglund.

519. Influence of treatment in a coronary care unit on prognosis in acute myocardial infarction. A controlled study in 271 cases. By Stefan Hofvendahl.

SUBJECT INDEX

(Supplements, see page V)

Diabetes mellitus

Endocrine glands

Gastro-intestinal tract

Heart

Hypertension

Infection

Intoxication

Kidney

Liver

Lung

Metabolism

Tumours

Urinary tract

Veins